

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number
WO 02/10767 A2

- (51) International Patent Classification⁷: **G01N 33/68**
- (21) International Application Number: **PCT/US01/23717**
- (22) International Filing Date: **26 July 2001 (26.07.2001)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
09/626,196 **28 July 2000 (28.07.2000)** **US**
- (63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US **09/626,196 (CIP)**
Filed on **28 July 2000 (28.07.2000)**
- (71) Applicant (for all designated States except US): **THE ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND [US/US]; 1430 Tulane Avenue, SL 13, New Orleans, LA 70112-2699 (US).**
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **TOBEY, Nelia, A. [US/US]; 5801 Walnut Creek, #D228, River Ridge, LA 70123 (US). ORLANDO, Roy, C. [US/US]; 1107 S. Peters Street, #122, New Orleans, LA 70130 (US).**
- (74) Agents: **SHORTLE, Rebecca et al.; Morrison & Foerster LLP, 755 Page Mill Road, Palo Alto, CA 94304-1018 (US).**
- (81) Designated States (national): **AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.**
- (84) Designated States (regional): **ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).**
- Published:**
— *without international search report and to be republished upon receipt of that report*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 02/10767 A2

(54) Title: **ASSAY METHODS FOR IDENTIFYING COMPOUNDS WHICH MAY PROTECT STRATIFIED SQUAMOUS EPITHELIUM AGAINST DAMAGE BY NOXIOUS SUBSTANCES**

(57) Abstract: Novel assay methods for screening compounds or identifying compounds useful for treating gastroesophageal disease (GERD) are described, which involve determining the level of or presence of an interaction between the test compound and a polypeptide sequence comprising a portion of the extracellular domain of the junctional protein E-cadherin or a related polypeptide sequence.

**ASSAY METHODS FOR IDENTIFYING COMPOUNDS WHICH MAY
PROTECT STRATIFIED SQUAMOUS EPITHELIUM AGAINST
DAMAGE BY NOXIOUS SUBSTANCES**

5 STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER
 FEDERALLY SPONSORED RESEARCH

This invention was made in part during work supported by a grant from the National Institutes of Health (NIH) ROI DK 36013. The government has certain rights in this invention.

10 TECHNICAL FIELD

The field of this invention relates to assay methods for the identification of compounds which may protect stratified squamous epithelial tissue against injury by noxious substances. More specifically, the field of the invention includes methods for identifying compounds which may be useful in the treatment of conditions associated with gastric reflux, more particularly, diseases such as gastroesophageal reflux disease (GERD).

BACKGROUND ART

Gastroesophageal Reflux Disease

Gastroesophageal reflux (GER) is the effortless movement of gastric contents from the stomach to the esophagus. It is a physiologic process, occurring in everyone, many times a day throughout life and without symptoms or signs of tissue injury. However, beginning at about 35-40 years old, GER is increasingly associated with the symptom of heartburn and morphologic injury to the esophagus. Indeed, a Gallup poll reported that up to 44% of adult Americans experience heartburn at least monthly and up to 10% have heartburn daily. The proximate cause for the transition from GER to gastroesophageal reflux disease (GERD) is the ability of noxious materials from the stomach to contact the esophagus with sufficient duration to result in damage to the epithelium. Moreover, it is well established that the major injurious substance

within the refluxate is gastric acid, though the latter may be aided by the presence of pepsin within the refluxate.

The damage resulting from acid exposure can be demonstrated morphologically and/or physiologically by changes in potential difference (PD), electrical resistance (R), or permeability to ions and molecules (Chung, R.S. *et al.* 5 (1975) *Am. J. Physiol.* 229(2):496-500; Dodds, W.J. *et al.* (1970) *Invest Radiol* 5:209-219; Harmon, J.W. *et al.* (1981) *Dig Dis Sci* 26:65-72; Kidder, J.W. *et al.* (1983) *J Lab Clin Med* 102:477-486; Kivilaakso, E. *et al.* (1980) *Surgery* 87:280-285; Salo, J. *et al.* (1982) *Surgery* 92:61-68). In esophageal epithelium the decline in R, in the absence of extensive tissue necrosis, is a direct reflection of increases in paracellular permeability as evidenced by a correlation between the decline in R with an increase in mannitol flux (Orlando, R.C. *et al.* (1981) *J Clin Invest* 68:286, Orlando, R.C. *et al.* (1984) *Am J Physiol* 246:G718-G724).

The pathophysiology of acid injury has been most thoroughly explored using the rabbit esophageal epithelium as a model for the human esophageal epithelium. Both human and rabbit esophageal epithelia are Na⁺-absorbing 'electrically-tight' stratified squamous epithelia. Each junctional complex consists of tight junctions with a few strands and an intercellular glycoprotein matrix. Moreover, rabbit and human esophageal epithelia both respond to high luminal acidity with a time-dependent biphasic change in transepithelial PD. Further, individual squamous cells utilize a basolateral membrane, acid-extruding, Na⁺/H⁺ exchanger (NHE-1 isotype) as a principal mechanism for regulation of intracellular pH (pHi) (Orlando, R.C. *et al.* In: Castell, D.O. *et al.*, eds. The Esophagus (3rd Edition). Philadelphia: Lippincott Williams & Wilkins, 1999, 409-419, and Orlando, R.C., *et al.* In: Orlando, R.C., *et al.*, eds. Gastroesophageal Reflux Disease. Marcel Decker, *in press*).

Key observations in humans that support a similar pathogenesis for acid reflux-induced injury to human esophageal epithelium as defined in the rabbit model are: 1) luminal perfusion *in vivo* with high concentrations of HCl (Bernstein test) produces a similar biphasic pattern in esophageal PD (Orlando, R.C. *et al.* (2000) *Am. J. Med.* 108 (4A):104S-108S, and Orlando, R.C. *et al.* In: Allen, A. *et al.*, eds. Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract. New York:

Raven Press, 1984, 75-79); and, 2) patients with both erosive and nonerosive GERD have the same morphologic hallmark of early acid damage to esophageal epithelium, *i.e.* dilated intercellular spaces. Tobey, N.A. *et al.* (1996) *Gastroenterology* 111:1200-1205.

5 Previous work has shown that sodium sulfate has a cytoprotective effect in acid exposed rabbit esophagus (Tobey, N.A. *et al.* (1986) *Am J Physiol* 251 (Gastrointest. Liver Physiol. 14):G866-G869). In U.S. Patents Numbers 5,374,537 and 5,189,056 it was alleged that protection of stratified squamous epithelia against damage from noxious luminal substances, *e.g.* HCl, was afforded by chemical
10 compounds having one of the following reactive groups in their molecule: $X-SO_3^-$, where X represents oxygen or carbon, and XO_4^- or $X_2O_7^-$, where X represents an element from Group VIb of the periodic table or sulfur. The compounds which were shown to provide protection against injury to stratified squamous epithelia included 4-acetamido-4'-isothiocyano-2,2'-stilbene disulfonate (SITS), 8-anilino-naphthalene-1-sulfonate (ANS), dinitrodisulfonic acid stilbene (DNDS), sulfonazo III, 4,4'-
15 diisothiocyano-2,2'-stilbene disulfonate (DIDS), bromophenol blue, ethane disulfonate, 1,3-benzene disulfonate, sucrose octasulfate (SOS), dextran sulfate, sodium chromate, sodium dichromate, sodium molybdate, sodium tungstate and sodium sulfate. Labeaga *et al.* disclose dosmalfate as a gastrointestinal and
20 esophageal cytoprotective agent (Labeaga, L. *et al.* (2000) *Drugs of Today* 2000, Vol. 36, Suppl. A:59-66). Sulfate ester compounds useful for treating and/or preventing gastroesophageal reflux disease (GERD) are disclosed in pending U.S. Provisional Patent Application titled "Methods For Protection Of Stratified Squamous Epithelium Against Injury By Noxious Substances And Novel Agents For Use Therefor" to
25 Hudson *et al.*, including disodium 3-(4-isothiocyantophenyl)-3 phenoxypropane-1,2-disulfate (CDDD-1192) and disodium 3-(4-isothiocyantophenoxy)-3-(4-isothiocyantophenyl)propane-1,2-disulfate (CDDD-1193).

In vitro methods for identification of potential agents useful for protection of stratified squamous epithelia against damage from noxious substances are disclosed in
30 U.S. Patent Nos. 5,374,537 and 5,189,056, Tobey, N.A. *et al.* (1986) *Am J Physiol* 251:G866-G869, and Orlando, R.C. *et al.* (1987) *Gastroenterology* 93:352-361.

There is a continued need for agents for treating gastroesophageal reflux disease (GERD) and other conditions associated with the exposure of stratified squamous epithelial tissue to noxious substances. There is a corresponding need for methods for identification of compounds which may protect stratified squamous epithelial tissue from injury resulting from exposure to noxious substances.

E-cadherin

E-cadherin belongs to the classical cadherin family of proteins, a subfamily of the cadherin superfamily of proteins. Classical cadherins are characterized as Ca^{2+} dependent glycosylated proteins consisting of an N-terminal extracellular domain, a single transmembrane domain and C-terminal cytosolic domain. Plott, R.T. *et al.* (1994) *J Invest Dermatol* 103:168-172. Cadherins are known to mediate cell-cell interactions, such as adhesion. E-cadherin in particular is implicated in the defense of cells against invasive mechanisms, such as in neoplasia, as well as playing an important role in embryonic development and tissue morphogenesis. Bussemakers, M.J. *et al.* (1993) *Molec Biol Reports* 17:123-128; Swami, S. *et al.* (1995) *Am J Gastroenterology* 90:1808-1813; Takeichi, M. (1991) *J Cell Biol* 75:464-474.

The polynucleotide encoding human E-cadherin has been isolated and characterized. Human E-cadherin cDNA has an open reading frame of 882 amino acids (SEQ ID NO:1) and a 3' non-coding region of 2035 nucleotides, and has an overall homology with mouse E-cadherin of 78%, and with the chicken E-cadherin related sequence, L-CAM, of 65%. There are few evident evolutionary changes across species, and those that are present are conservative amino acid substitutions. The transmembrane domain of the human and mouse proteins are identical, and the cytoplasmic domain, important for the regulation of the cell-cell binding function of the extracellular domain, is almost identical (with only conservative amino acid substitutions). Bussemakers, M.J. *et al.* (1993) *Molec Biol Reports* 17:123-128.

Processing of the human E-cadherin precursor results in a mature protein with amino acids numbered from 155 to 882. Analysis of human E-cadherin

suggests an extracellular domain of approximately 553 amino acids (amino acid 155 to approximately amino acid 707) with five internally repeated domains of approximately 110 amino acids which are involved in Ca^{2+} binding. The corresponding nucleotide bases for the extracellular domain are from approximately nucleotide 557 to approximately nucleotide 2215 (nucleotide numbering according to Bussemakers, M.J. *et al.* (1993) *Molec Biol Reports* 17:123-128). The five extracellular repeat domains (EC1-EC5) have been reported to correspond to amino acid residues approximately: EC1 - 155-262; EC2 - 263-375; EC3 - 376-486; EC4 - 487-593; EC5 - 594-697, according to the sequence reported in GenBank accession number P12830 (<http://www.ncbi.nlm.nih.gov/PubMed/>). EC1-EC4 have from 19-29% internal sequence identity, while EC5 is less related to the other four subdomains with only 10-16% sequence identity. Alattia, J.R. *et al.* (1999) *Cell Mol Life Sci* 55:359-367.

Several structural studies of isolated portions of the extracellular domain of E-cadherin have also been carried out, including X-ray crystallography and NMR studies. Alattia, J.R. *et al.* (1999) *Cell Mol Life Sci* 55:359-367 and references cited therein; Overduin, M. (1996) *J Biol NMR* 7:173-189; Gumbiner, B.M. *et al.* (1996) *Cell* 84:345-357. The basic structure of the intra-domain repeats appear to be a fold consisting of a seven stranded β -sheet with the N and C termini of the fragment at opposite ends of the fold. The fold resembles a barrel and contains two small helices. It is thought that the N-terminal tip of the molecule is involved in cell adhesion, with the adhesion specificity residing in the HAV region in domain 1 of E-cadherin. Overduin, M. *et al.* (1995) *Science* 267:386-389; Takeichi, M. (March 22, 1991) *Science* 251:1451-1455; Pertz, O. *et al.* (1999) *EMBOJ* 18:1738-1747; Geiger *et al.* (1992) *Ann Rev Cell Biol* 8:307-332.

E-cadherin can also be characterized by its Ca^{2+} dependency. In the presence of Ca^{2+} , E-cadherin is resistant to cleavage by trypsin, while in the absence of Ca^{2+} , the protein can be cleaved by trypsin, resulting in an approximately 80kD fragment, which corresponds to the extracellular domain.

These findings are consistent with the presence of Ca^{2+} binding regions in the extracellular domain. Plott, R.T. *et al.* (1994) *J Invest Dermatol* 103:168-172; Gumbiner, B.M. *et al.* (1986) *J Cell Biol* 102:457-468.

5 Cadherins have been identified as the major adhesion receptors of the zonula adherens junctions of epithelia. In addition, the expression and activity of E-cadherin is vital for the tight association of cells and the maintenance of the macromolecular structure of epithelial tissue. In the absence of functional E-cadherin, intercellular adhesion is not maintained, despite the presence of other adhesion and junctional proteins. Gumbiner, B.M. (1996) *Cell* 84:345-357.
10 Calcium also appears necessary for cell-cell adhesion. Alattia, J.R. *et al* (1999) *Cell Mol Life Sci* 55:359-367.

Studies of kidney epithelial tissue reveal that antibodies which can bind E-cadherin also inhibit the recovery of R in junctions made permeable by the removal of Ca^{2+} from the surrounding solution. The antibody recognizes a 118 kD
15 polypeptide from kidney epithelium that is the same as or related to the cell adhesion molecule Uvomorulin/E-cadherin. Additionally, after the junction has been made permeable in the absence of Ca^{2+} , incubation with trypsin leads to poor recovery of R upon the restoration of Ca^{2+} . The lack of recovery in these cells compared to cells not treated with trypsin or cells treated with trypsin in the
20 presence of Ca^{2+} , without concomitant loss of cells, suggests that the lack of recovery is due to the degradation by trypsin of a cell-surface protein. Gumbiner, B.M. *et al.* (1986) *J Cell Biol* 102:457-468. The antibody isolated in the experiments described above also recognized the approximately 80kD fragment of E-cadherin resulting from E-cadherin cleaved by trypsin in the presence of Ca^{2+} .

25 ***Desmosomal Subfamily***

Desmocollin 3, which belongs to the desmosomal subfamily of the cadherin superfamily, is a 896 residue protein, with the extracellular domain having 555 amino acids numbered 136-690, as described in GenBank accession number Q14574 (SEQ ID NO:5).

The desmogleins, a subfamily of the desmosomal cadherins, share amino acid homologies in both the cytoplasmic and extracellular domains with the classical cadherins. Desmoglein 1 is a 1049 residue protein, with the extracellular domain having 499 amino acids numbered 50-548, as described in GenBank accession number IJHUG1 (SEQ ID NO:2) (http://www.ncbi.nlm.nih.gov/PubMed/). Desmoglein 2 is a 1117 residue protein, with the extracellular domain having 560 amino acids numbered 49-608, as described in GenBank accession number Q14126 (SEQ ID NO:3). Desmoglein 3 (also known as pemphigus vulgaris antigen) is a 999 residue protein, with the extracellular domain having 566 amino acids numbered 50-615, as described in GenBank accession number IJHUG3 (SEQ ID NO:4). Desmoglein 3 has been shown to have an overall sequence identity of 33% to human E-cadherin. Plott, R.T. *et al.* (1994) *J Invest Dermatol* 103:168-172. Additionally, alignment of the human E-cadherin sequence with extracellular subdomains 1-5 of desmoglein 3 result in sequence identities between the two proteins of EC1, 31%, EC2, 45%, EC3, 33%, EC4, 30% and EC5, 28%. Plott, R.T. *et al.* (1994) *J Invest Dermatol* 103:168-172.

Other Polypeptides With Substantial Homology to E-cadherin

The HA(V/N) region of HA1 chain influenza strain A hemagglutinins show substantial sequence homology with cadherins. Blaschuk *et al.* (1990) *J Mol Biol* 211:679-682. The hemagglutinins examined were split into two groups, with the overall HA(V/N) domain of group 1 hemagglutinins (SEQ ID NOS:6-13) having 25% of residues that are identical to the consensus sequence for the cadherin family, and an additional 25% of residues being either partially conserved or homologous according to the Dayhoff matrix. For group 2 hemagglutinins (SEQ ID NOS:14-21) the percent identity and homology are 13% and 30%, respectively.

HAV domains of fibroblast growth factor receptors (FGF-R) (SEQ ID NOS:22-25) showed sequence identity of 25% with cadherins with an additional

33% being partially conserved or homologous. Byers, S. *et al.* (1992) *Dev Biol* 152:411-414.

All references cited herein are hereby incorporated by reference in their entirety.

5

DISCLOSURE OF THE INVENTION

One method of the present invention comprises assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising, in order: a) contacting a polypeptide with a compound; wherein the polypeptide is selected from the group consisting of an extracellular
10 portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted within the extracellular domain of SEQ ID NO:1; and b) determining whether the compound binds to the polypeptide; wherein binding of the compound to the polypeptide indicates that the compound may protect stratified squamous epithelia from damage by a noxious substance.

15

Another method of the present invention comprises assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising, in order: a) contacting a polypeptide with a labeled cytoprotective agent; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at
20 least 13% sequence identity to a sequence depicted within the extracellular domain of SEQ ID NO:1; b) contacting the polypeptide with a compound; and c) comparing binding of the agent to the polypeptide contacted with the compound to a control conducted where the polypeptide is contacted with the agent and is not contacted with the compound; wherein a decrease in binding of the agent to
25 the polypeptide contacted with the compound compared to the control indicates that the compound may protect stratified squamous epithelia against damage by a noxious substance.

30

Another method of the present invention comprises identifying a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising, in order: a) contacting a polypeptide with a

compound; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted within the extracellular domain of SEQ ID NO:1; b) contacting the polypeptide with a monoclonal antibody to the polypeptide; and c) comparing the amount of antibody binding to the polypeptide contacted with the compound to the amount of antibody binding to a control; wherein a decrease in the amount of antibody binding to the polypeptide which has been contacted with the compound compared to the control indicates that the compound may protect stratified squamous epithelia from damage by a noxious substance.

Another method for use in the present invention comprises assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising, in order: a) contacting a polypeptide with a compound; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted within the extracellular domain of SEQ ID NO:1; b) determining the stability of the polypeptide; and c) comparing the stability of the polypeptide contacted with the compound to the stability of a control; wherein a greater stability for the polypeptide contacted with the compound compared to the control indicates that the compound may protect stratified squamous epithelia from damage by a noxious substance.

Another method for use in the present invention comprises assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising, in order: a) contacting a polypeptide with the compound; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted within the extracellular domain of SEQ ID NO:1; b) contacting the polypeptide with a noxious substance; and c) determining the stability of the polypeptide; and d) comparing the stability of the polypeptide contacted with the compound and the noxious substance to the stability of a control; wherein if the stability of the polypeptide contacted with the

compound and noxious substance is greater than that of the control, the compound may protect stratified squamous epithelium against damage by the noxious substance.

5 An additional method of the present invention comprises assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising: a) contacting a polypeptide with the compound; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted within the extracellular domain of SEQ ID NO:1; b)
10 contacting the polypeptide with a noxious substance; c) determining the amount of fragmentation of the polypeptide; and d) comparing the amount of fragmentation of the polypeptide to a control; wherein a decrease in the amount of fragmentation of the polypeptide contacted with the compound compared to the control indicates that the compound may protect stratified squamous epithelia
15 from damage by a noxious substance.

MODES FOR CARRYING OUT THE INVENTION

Definitions

As used herein, the term "compound" means a biological or chemical compound such as a simple or complex organic or inorganic molecule, a peptide,
20 a protein or an oligonucleotide. A vast array of compounds can be synthesized, for example oligomers, such as oligopeptides and oligonucleotides, and synthetic organic compounds based on various core structures, and these are also included in the term "compound". In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like.
25 Compounds can be tested singly or in combination with one another. "Compounds" may also be referred to as "drugs" or "pharmaceutical compounds" or "pharmaceutical candidates".

A "noxious substance", as used herein, refers to a substance which causes injury to stratified squamous epithelium *in vivo* or *in vitro*. Examples of such

“noxious substances” include acids or other substances, including, but not limited to, gastric acid, HCl, N-acetylcysteine, pepsin, acid-pepsin, gastrointestinal fluid, or other irritant which contacts epithelial tissue. The term “noxious substance” may also be used to refer to a combination of noxious substances, such as two or more noxious substances.

An “individual” is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, farm animals, sport animals, primates, rodents and pets, including pigs, sheep, horses, cows, rabbits, mice and rats.

A “susceptible individual” is an individual who suffers from or is likely to or predisposed to suffer from a condition(s) which causes contact or exposure between their epithelial tissues and noxious substances. In humans these conditions may include, for example, gastroesophageal reflux disease (GERD), heartburn, indigestion, laryngitis, Barrett’s Esophagus, or pharyngitis. In animals these conditions may include, for example, peptic ulcers, gastritis, erosions, perforation and hemorrhage.

The terms “injury” or “damage” as used herein are interchangeable and refer to the cellular and morphological manifestations and symptoms resulting from exposure to or contact with a noxious substance. Indications of injury or damage include, but are not limited to, acid-induced decline in PD, acidification of the intracellular or intercellular space, prominently dilated intercellular spaces, increased permeability of intercellular junctions to noxious substances; macroscopic erosions and ulceration of epithelial tissue, cell edema, inflammation and necrosis, peptic stricture, Barrett’s Esophagus, heartburn, laryngitis, pharyngitis, hoarseness and sore throat.

The terms “stratified squamous epithelium”, “stratified epithelium”, “epithelia” and “epithelium” are interchangeable as used herein. Sources of stratified squamous epithelium include, for example, buccal, oropharyngeal, esophageal and laryngeal epithelium, rumen and forestomach.

The polypeptide known as “E-cadherin” is also known as “CAM 120/80”, “L-CAM”, “Arc-1” and “Uvomorulin”. Unless otherwise noted, these terms may

be used interchangeably herein. A preferred form of E-cadherin is the human sequence.

The term "cadherin" refers to a protein which is a member of the cadherin superfamily of proteins. The cadherin superfamily includes, for example, the subfamily of classical cadherins, specific examples of which include E-cadherin, N-cadherin, and P-cadherin, and the subfamily of desmosomal proteins, specific examples of which include desmogleins 1, 2 and 3, and desmocollin 3. Natural sources of cadherins may be found in vertebrates, including humans, farm animals, sport animals, primates, rodents and pets, for example, chickens, pigs, sheep, horses, cows, rabbits, mice and rats.

The term "extracellular domain" refers to the region of a protein that is not embedded in the membrane and exists outside the cellular membrane.

The term "extracellular portion" refers to an isolated polypeptide comprising at least 5 amino acid residues of an extracellular domain of a protein. For example, the term "extracellular portion" may encompass smaller domains or subdomains, such as the 5 repeat regions of approximately 110 residues within the extracellular domain of E-cadherin. The isolated polypeptide may be purified from a natural source or maybe obtained by synthetic methods known in the art.

A "cytoprotective agent" is an agent which protects stratified squamous epithelia from damage by a noxious substance. Classes of compounds which include examples of such agents include sulfonates, sulfate esters and inorganic tetrahedral-shaped divalent oxyanions. Specific examples include SITS, SOS, CDDD-1192 and CDDD-1193.

The term "protection" as used herein refers to the reduction of damage by a noxious substance to epithelium which has been contacted with an agent *in vivo* or *in vitro*. The protection provided by the agent may last for at least 10 minutes, at least 20 minutes, at least 30 minutes; at least 1 hour, at least 2 hours, at least 4 hours, at least 8 hours, at least 12 hours, at least 24 hours after contact with the compound.

The term "irreversible protection" refers to protection afforded by compounds, which, when contacted with the epithelium, are resistant to being

washed off the epithelium. Irreversible protection may be indicated by compounds, which for example, when contacted with the epithelium *in vitro*, continue to prevent or reduce acid-induced decline in R after at least one washing with a buffer such as normal Ringer solution.

5 The term "control" refers to an experiment conducted under the same conditions as the experiment to which it is being compared and is designed to measure the same experimental parameter. "Controls" may be used to establish typical values for the experimental parameter being measured under particular conditions. A "control" is not required to be run in parallel with the experiment in
10 question and may be run within hours, days, weeks or months of the experiment in question.

 An "antibody" (interchangeably used in plural form) is an immunoglobulin molecule capable of specific binding to a target, such as a polypeptide, through at least one antigen recognition site which is located in the
15 variable region of the immunoglobulin molecule. An antibody can be from any source of animal capable of producing them, for example, mouse, rat, rabbit, primate, or human antibodies. As used herein, the term encompasses not only intact antibodies, but also fragments thereof (such as Fab, Fab', F(ab')₂, Fv, single chain (ScFv)), mutants thereof, fusion proteins, chimeric antibodies, humanized
20 antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity. The term "antibody" includes polyclonal antibodies and monoclonal antibodies.

 The terms "polypeptide", "oligopeptide", "peptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The
25 polymer may be linear or branched, it may comprise modified amino acids, and it may be assembled into a complex of more than one polypeptide chain. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, by disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as
30 conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid

(including, for example, unnatural amino acids, etc.), as well as other modifications known in the art.

5 In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an N-terminal to C-terminal direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide. A "partial sequence" is a linear sequence of part of a polypeptide which is known to comprise additional residues in one or both directions.

10 An "isolated" or "purified" polynucleotide, polypeptide, antibody or cell is one that is substantially free of the materials with which it is associated in nature. By substantially free is meant at least 50%, preferably at least 70%, more preferably at least 80%, and even more preferably at least 90%, at least 95%, at least 99% free of the materials with which it is associated in nature. As used herein, an "isolated" polynucleotide or polypeptide also refers to recombinant
15 polynucleotides or polypeptides, which, by virtue of origin or manipulation: (1) are not associated with all or a portion of a polynucleotide or polypeptide with which it is associated in nature, (2) are linked to a polynucleotide or polypeptide other than that to which it is linked in nature, or (3) does not occur in nature, or (4) in the case of polypeptides arising from expression of recombinant
20 polynucleotides. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichments of the embodiments of the
25 compositions used in the methods of this invention are increasingly more preferred. Thus, for example, a 2-fold enrichment is preferred, 10-fold enrichment is more preferred, 100-fold enrichment is more preferred, 1000-fold enrichment is even more preferred. A substance can also be provided in an isolated state by a process of artificial assembly, such as by chemical synthesis or
30 recombinant expression.

A substance is said to be "selective" or "specific" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances.

As used herein, "naturally occurring," "native," or "wild type" refers to endogenous E-cadherin polynucleotides and the E-cadherin protein(s) expressed thereby, including alleles and allelic forms of the protein(s). These terms include full-length and processed polynucleotides and polypeptides. Processing can occur in one or more steps, and these terms encompass all stages of processing. For instance, polypeptides having or lacking a signal sequence may be used. "Non-naturally occurring", "non-native", or "non-wild type" refer to all other E-cadherin polynucleotides and polypeptides.

The term "stability" refers to the measure of the ability of a protein or fragment thereof to maintain its native conformation and/or activity. Stability may be measured by techniques well known in the art.

A polypeptide or polypeptide region having a certain percentage (for example, 10%, 13%, 20%, 50%, 70%, 75%, 80%, 85%, 90%, or 95%) of "sequence identity" to another sequence means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and percent identity may be determined using software programs known in the art, for examples those described in *Short Protocols in Molecular Biology* (John Wiley and Sons., eds., 1999).

Sequence identity is generally determined after sequences have been aligned to maximize the number of matches between the sequences. A variety of alignment programs and algorithms are known in the art. One suitable program for polypeptide sequences is blastn which uses a heuristic search algorithm (BLAST) that is also useful for sequence alignments. See, for example, Altschul, S.F. *et al.* (1990) *J Mol Biol* 215:403-410. BLAST programs may be accessed through the National Center for Biotechnology Information (NCBI) internet site

(www.ncbi.nlm.nih.gov/BLAST/). The default parameters of the NCBI blastn programs are suitable for generating sequence alignments for the sequences described herein. Other suitable alignment programs are commercially available. The GAP program from Genetics Computer Group, Inc. (GCG; Madison, WI), for
5 example, uses the algorithm of Needleman and Wunsch (1970, *J Mol Biol* 48:443-453) to generate the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps. For another example, the BESTFIT program from GCG uses the local homology algorithm of Smith and Waterman (1981, *Advances in Applied Mathematics* 2:482-489) to generate the
10 optimal alignment of the best segment similarity between two sequences through the insertion of gaps to maximize the number of matches. When identifying the best alignment, the default gap penalty is 50 and the default gap extension penalty is 3 for the GAP and BESTFIT programs.

Chemical terms, unless otherwise defined, are used as known in the art.
15 "A", "an" and "the" include plural references unless the context clearly dictates otherwise.

General Techniques

Techniques for isolation and manipulation of cadherin proteins may be found in, for example, Gumbiner, B.M. (1996) *J Cell Biol*:102:457-468, Gumbiner, B.M. (1996) *Cell* 84:345-357, Takeichi, M. (1977) *J Cell Biol* 75:464-474, and
20 Takeichi, M. (1988) *Development* 102:639-655. Techniques and protocols for manipulating epithelial tissue and using Ussing chambers may be found in the art and in particular in: U.S. Patent No. 5,374,537; U.S. Patent No. 5,189,056; Abdunour-Nakhoul, S. *et al* (2000) *Am J Physiol* 278:G113-G120, Carney, C.N. *et al* (1981) *Lab Invest* 45:198-208, Orlando, R.C. *et al* (1981) *J Clin Invest* 68:286, Orlando, R.C. *et al* (1984) *Am J Physiol* 246:G718-G724, Khalbuss, W.E. *et al* (1995) 108:662-672, Tobey, N.A. *et al* (2000) *J Invest Med* 108(4A):144A, Tobey, N.A. *et al* (1999) *Gastroenterology* 116:A334, Orlando, R.C. *et al.* (2000) *Am J Med* 108(4A):104S-108S, Tobey, N.A. *et al.* (1989) *Gastroenterology*
25 96:1466-1477, Orlando, R.C. *et al* (1984) *New York: Raven Press*:75-79, Orlando,
30

R.C. *et al* (1982) *Gastroenterology* 83:1026-1032, and Tobey, N.A. *et al* (1996) *Gastroenterology* 83:1026-1032.

Methods of Assaying for Compounds Which May Protect Stratified Squamous Epithelia

5 As described in the following examples, we have identified the mechanism
by which cytoprotective agents, such as SITS or SOS, protect epithelial tissue
from exposure to noxious substances. Without being bound by any particular
theory, it appears that this mechanism comprises the binding of the agent to the
10 extracellular domain, portion, or a region or fragment thereof, of E-cadherin, a
junctional protein found in the zonula adherens of epithelial tissue. Based upon
this discovery, novel assay techniques have been designed to screen for
compounds which will bind to a polypeptide selected from the group consisting of
an extracellular portion of a cadherin or a peptide sequence having at least 13%
sequence identity to a sequence depicted within the extracellular domain of E-
15 cadherin (SEQ ID NO:1), where binding is indicative of a compound which may
protect stratified squamous epithelial from damage or injury by noxious
substances, such as damage stemming from acid reflux.

 Accordingly, one method of the present invention comprises assaying for a
compound which may protect stratified squamous epithelia from damage by a
20 noxious substance, comprising, in order: a) contacting a polypeptide with a
compound; wherein the polypeptide is selected from the group consisting of an
extracellular portion of a cadherin or a peptide sequence having at least 13%
sequence identity to a sequence depicted within the extracellular domain of SEQ
ID NO:1; and b) determining whether the compound binds to the polypeptide;
25 wherein binding of the compound to the polypeptide indicates that the compound
may protect stratified squamous epithelia from damage by a noxious substance.

 An additional embodiment further comprises the step of: c) comparing the
binding of the compound to the polypeptide with the binding of a cytoprotective
agent to the polypeptide; wherein a greater than or equal level of binding by the
30 compound indicates that the compound may protect stratified squamous epithelia
from damage by the noxious substance.

Another method of the present invention comprises assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising, in order: a) contacting a polypeptide with a labeled cytoprotective agent; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted within the extracellular domain of SEQ ID NO:1; b) contacting the polypeptide with a compound; and c) comparing binding of the agent to the polypeptide contacted with the compound to a control conducted where the polypeptide is contacted with the agent and is not contacted with the compound; wherein a decrease in binding of the agent to the polypeptide contacted with the compound compared to the control indicates that the compound may protect stratified squamous epithelia against damage by a noxious substance.

The determination of protein to substrate binding, in this context, binding to either compound or cytoprotective agent, is well known in the art, and need not be discussed in detail herein. Exemplary techniques for determining binding include, for example, labeling the substrate (*i.e.* the compound or agent binding to the protein) with, for example, a radioactive isotope or fluorescent label. Examples of fluorescent labels include, but are not limited to, biotin, fluorescein, Texas red, Lucifer yellow, and rhodamine. Other labeling methods include electron dense tracers, such as, alkaline phosphatase, horseradish peroxidase and glucose oxidase.

Another method of the present invention comprises identifying a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising, in order: a) contacting a polypeptide with a compound; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted within the extracellular domain of SEQ ID NO:1; b) contacting the polypeptide with a monoclonal antibody to the polypeptide; and c) comparing the amount of antibody binding to the polypeptide contacted with the compound to the amount of antibody binding to a control;

wherein a decrease in the amount of antibody binding to the polypeptide which has been contacted with the compound compared to the control indicates that the compound may protect stratified squamous epithelia from damage by a noxious substance.

5 In one embodiment of the above method, the control may comprise contacting the polypeptide with the monoclonal antibody or may comprise contacting the polypeptide with a cytoprotective agent and then contacting the polypeptide with the monoclonal antibody.

10 Another method for use in the present invention comprises assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising, in order: a) contacting a polypeptide with a compound; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted with in the extracellular domain of SEQ
15 **ID NO:1**; b) determining the stability of the polypeptide; and c) comparing the stability of the polypeptide contacted with the compound to the stability of a control; wherein a greater stability for the polypeptide contacted with the compound compared to the control indicates that the compound may protect stratified squamous epithelia from damage by a noxious substance.

20 In one embodiment, controls for the method described above includes where the control comprising the polypeptide is not contacted with the compound or where the polypeptide is contacted with a cytoprotective agent and is not contacted with the compound.

25 Another method for use in the present invention comprises assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising, in order: a) contacting a polypeptide with the compound; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted within the extracellular domain of SEQ
30 **ID NO:1**; b) contacting the polypeptide with a noxious substance; and c) determining the stability of the polypeptide; and d) comparing the stability of the

polypeptide contacted with the compound and the noxious substance to the stability of a control; wherein if the stability of the polypeptide contacted with the compound and noxious substance is greater than that of the control, the compound may protect stratified squamous epithelium against damage by the noxious substance.

Controls for the above method may include, in certain embodiments, where a control comprising the polypeptide is not contacted with the compound but is contacted with the noxious substance or where the polypeptide is contacted with a cytoprotective agent and then contacted with the noxious substance.

While not wishing to be bound by any particular theory, it appears, from the data presented in the Examples, that the mechanism by which cytoprotective agents function is to maintain the stability of the junctional protein E-cadherin in the presence of noxious substances. While the overall stability of the protein is not enhanced by the binding of cytoprotective agents over that of the native protein, under, for example, denaturing conditions or in the presence of a noxious substance, native-like characteristics should be maintained, such as exemplified by conformation or biological activity (*e.g.* the maintenance of junctional impermeability).

The stability of the extracellular portion may be measured by techniques well known in the art, and need not be discussed herein. Examples of such techniques include, but are not limited to, calorimetric measurement, such as the determination of the temperature of denaturation, for example, digital scanning calorimetry, resistance to denaturation, as measured by, for example, titration against temperature, pH or chemical denaturants such as guanidine hydrochloride or urea. Stability may be measured under varying conditions of hypertonicity, hypotonicity, and concentrations of N-acetylcysteine, EMPIGEN® detergent (Calbiochem, San Diego, CA), bile acids and bile salts. Titrations may be monitored by a number of means, including HPLC (high performance liquid chromatography), FPLC (fast performance liquid chromatography), and spectroscopic or optical means, such as fluorescence. Additional examples of measures of stability include resistance to fragmentation by, for example, acid

cleavage or enzymatic digestion, including but not limited to enzymes such as pepsin, trypsin, chymotrypsin and concentrated acids such as hydrochloric acid, sulfuric acid, phosphoric acid, gastric acid and others. Fragmentation upon exposure to noxious substances occurring within the digestive system or testing analogues thereof, is also contemplated. Examples of such substances include acid, acid-pepsin and N-acetylcysteine. Examples of determining the degree of fragmentation are discussed below. Persons of skill in the art will recognize that numerous biophysical techniques are available to measure the stability of proteins, or portions thereof, with or without substrate (*i.e.* compound or agent) bound to the protein or protein fragment.

An additional method of the present invention comprises assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising: a) contacting a polypeptide with the compound; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted within the extracellular domain of **SEQ ID NO:1**; b) contacting the polypeptide with a noxious substance; c) determining the amount of fragmentation of the polypeptide; and d) comparing the amount of fragmentation of the polypeptide to a control; wherein a decrease in the amount of fragmentation of the polypeptide contacted with the compound compared to the control indicates that the compound may protect stratified squamous epithelia from damage by a noxious substance.

In certain embodiments, controls for the above-described method may include where the polypeptide is not contacted with the compound but is contacted with the noxious substance or where the polypeptide is contacted with a cytoprotective agent and then contacted with the noxious substance.

In the present embodiment, fragmentation is the result of exposure to a noxious substance, such as acid, N-acetylcysteine or acid-pepsin. The degree of fragmentation is indicative of the amount of protection afforded by the compound being assayed, where a decrease in fragmentation compared to a control indicates protection. Determination of the degree or amount of fragmentation may be

accomplished using techniques known in the art, exemplary techniques including electrophoresis, including SDS gel electrophoresis and capillary electrophoresis, isoelectric focusing, HPLC, FPLC, mass spectrometry, 2D-gels, amino acid analysis, and amino acid sequencing.

5 In general, for any of the methods of the present invention, controls are run such that they comprise the same polypeptide under the same experimental conditions, except that controls are not contacted with the compound which is being tested or are instead contacted with a cytoprotective agent, as specified. Controls need not be run in parallel to the experiment in question, and may consist
10 of prior experimentation done days, weeks or months prior to the experiment in question, that establishes typical values for a set of experimental conditions. Examples of particular cytoprotective agents for use in control experiments may be found, for example in U.S. Patent Nos. 5,374,537 and 5,189,056, and in pending U.S. Provisional Patent Application titled "Methods For Protection Of
15 Stratified Squamous Epithelium Against Injury By Noxious Substances And Novel Agents For Use Therefor" to Hudson *et al.* Preferred classes of compounds for use as cytoprotective agents include sulfate esters and sulfonates. Examples of preferred cytoprotective agents includes SITS, SOS and CDDD-1193 and CDDD-1192.

20 Experimental conditions may include, for example, pH, temperature, duration of contact with a noxious substance, concentration of noxious substance, concentration of polypeptide, number and duration of washes, composition of wash solutions and the like. Preferred experimental conditions include, for example, pH from about 1-14, temperature from about 0-100°C, duration of
25 contact with noxious substances from about 1 minute to 24 hours, duration of washes from about 1 minute to 24 hours; number of washes from about 1 to 20, concentration of noxious substances from about 1 μ molar to 1 molar, concentration of polypeptide from about 1 picomolar to 10 millimolar. More preferred experimental conditions may include, for example, pH from about 1-4,
30 pH from about 1-2.5, pH about 1.6, temperature from about 25-37°C, temperature of about 37°C, duration of contact with noxious substances from about 1 minute to

1 hour, duration of contact with noxious substances from about 1 to 30 minutes, duration of washes from about 1 to 5 minutes, number of washes from about 1 to 5, number of washes from about 3 to 4, concentration of HCl from about 10 millimolar to 100 millimolar, concentration of pepsin from about 0.1 to 10 mg/ml, concentration of bile salts from about 500 μ M to 1 mM, concentration of polypeptide from about 1 nanomolar to 10 micromolar, and concentration of polypeptide from about 1 micromolar to 10 millimolar. *In vitro* experiments may be run in physiologic buffers or serum. Wash solutions may be any aqueous vehicle. Preferred examples include water, saline, Ringer, HEPES buffer, Tris-buffer, saliva, or salivary ion composition solutions. The compound or agent may be contacted with the polypeptide, for example, for at least 30 seconds, at least 1 minute, at least 5 minutes, at least 10 minutes, at least 20 minutes, at least 30 minutes, at least 60 minutes, or at least 90 minutes.

Preferred compounds to be assayed include, but are not limited to, compounds selected from the group including sulfonates and sulfate esters.

As discussed in detail in the Examples, we have identified the extracellular domain of the zonula adherens junctional protein E-cadherin as the region where cytoprotective agents bind. In addition, monoclonal antibodies to desmoglein 1 and 2, and desmocollin 3, members of the cadherin superfamily of proteins, appear to bind to the extracellular domain of E-cadherin. While not wishing to be bound by a particular theory, this monoclonal antibody cross-reactivity is likely a result of the degree of homology or identity of the extracellular domains of these cadherin proteins, and suggests that compounds which bind the extracellular domain of E-cadherin will also bind the extracellular domains of similar proteins.

Members of the cadherin superfamily of proteins, and extracellular portions thereof, may be used as polypeptides in the novel assays techniques described herein. Preferred sequences include extracellular portions of proteins of the classical cadherin and desmosomal subfamilies. More preferred are extracellular portions from the proteins E-cadherin (SEQ ID NO:1), desmoglein 1 (SEQ ID NO:2), 2 (SEQ ID NO:3), or 3 (SEQ ID NO:4), and desmocollin 3 (SEQ ID NO:5). Even more preferred are extracellular portions from E-cadherin.

Particularly preferred are extracellular portions from E-cadherin residues 155-262, desmoglein 1 residues 52-157, desmoglein 2 residues 49-159, desmoglein 3 residues 52-157, and desmocollin 3 residues 136-243.

Additional peptides with sequence similarity to extracellular portions of E-cadherin may also be used as polypeptides for testing possible drug candidates or compounds and may be used in any of the methods of the invention described herein. Examples of such peptides include the HA(V/N) region of the HA1 chain of influenza A hemagglutinin, examples of which are shown in **SEQ ID NOS:6-21** and HAV region sequences from fibroblast growth factor receptor, examples of which are shown in **SEQ ID NOS:22-25** (Table 1).

Table 1. Sequences from HA(V/N) region of the HA1 chain of influenza A hemagglutinin and HAV region of fibroblast growth factor receptor		
Type	Sequence	SEQ ID NO
HMIVF	DKICLGHHAVSNGTKVNTLTERGVEVVNATE	6
HMIVH	QDLPGNDNNTATLCLGHHAVPNGTLVKTTDDQIEVTNATE	7
HMIV6	QNLPGNDNSTATLCLAHHAVPNGTLVKTTNDQIEVTNATE	8
HMIVV	QDLPGNDNSTATLCLGHHAVPNGTLVKTTNDQIEVTNATE	9
HMIVDU	QDLPGNDNSTATLCLGHHAVPNGTIVKTTDDQIEVTNATE	10
HMIVH3	QYLPGNDNSTATLCLGHHAVPNGTLVKTTNDQIEVTNATE	11
HMIVH7	DKICLGHHAVANGTKVNTLTERGIEVVNATE	12
HMIV10	DKICLGHHAVPNGIIVKTLTNEKEEVTNATE	13
HMIVH6	DKICIGYHANNSTTQIDTILEKNVTVTHSVLELLEN	14

HMIV5	DTICIGYHANNSTDVDTIFEKNVAVTHSVNLLED	15
HMIV	DTICIGYHANNSTDVDTVLEKNVTVTHSVNLLE D	16
HMIVUR	DTICIGYHANNSTDVDTVLEKNVTVTHSVNLLE D	17
HMIV2	DQICIGYHANNSTEKVDITLERNVTVTHAKDILE K	18
HMIVH2	DQICIGYHANNSTEKVDITLERNVTVTHAKDILEK	19
HMIVH5	DQICIGYHANNSTEQVDITIMEKNVTVTHAQDILE K	20
HMIVN1	DTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLE D	21
chick bFGF-R	EKMEKKLHAVPA	22
mouse FGF-R	EKMEKKLHAVPA	23
frog FGF-R	EKMEKKLHAVPA	24
mouse KGF-R	EKMEKRLHAVPA	25

Preferably, the peptide for use in the assay methods described herein will have at least 13%, at least 19%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70% sequence identity with an extracellular portion of E-cadherin as depicted in SEQ ID NO:1.

In certain embodiments, the polypeptide may comprise the sequence, or fragment thereof, of the sequences described above, wherein the fragment of the sequence is at least 5, at least 6, at least 10, at least 25, at least 50, at least 100, at least 110, at least 120; at least 150, at least 200, at least 220, or at least 300 amino acids in length.

In a preferred embodiment, the human sequence of the protein will be used. In another embodiment, other variants may be used in the methods of the invention described herein, including those from farm animals, pet, rodents, primates and sport animals, such as chicken, rabbit, rat, mouse, horse, cow, sheep, and pig sequences.

Embodiments of any of the assay methods described above may further comprise the following steps, in order: contacting a sample comprising stratified squamous epithelial tissue with the compound; contacting the sample with a noxious substance; and determining if the epithelia is damaged. These steps further the level of screening of the compound and therefore increase the likelihood of identification of an effective drug candidate. Techniques for performing the above-listed steps may be found in the art, particularly in: Tobey, N.A. *et al* (1986) *Am J Physiol* 251:G866-G869, Orlando, R.C. *et al* (1987) *Gastroenterology* 93:352-361, U.S. Patent No. 5,374,537, and U.S. Patent No. 5,189,056.

In particular embodiments, stratified squamous epithelial tissue may be obtained from esophageal, oral cavity, cheek, pharynx, larynx, or vocal cord tissue from an individual. A preferred embodiment is where the epithelia is obtained from rabbit esophageal tissue. Certain additional embodiments include tissue from rats, pigs, horses, or sheep and may also be selected from the forestomach or rumen of such animals. Additional embodiments include human epithelial tissue which may be obtained, for example from autopsy, surgical or biopsy specimens.

In vivo, epithelial tissue is exposed to gastrointestinal fluid, which comprises, in part, acid, acid-pepsin and other constituents, such as rennin, lipase, bile salts and acids, pancreatic enzymes such as trypsin, elastase, chymotrypsin and amylase, and hydrochloric acid (HCl). *In vitro* experiments performed on stratified squamous epithelial tissue in Ussing chambers, as discussed previously, for example in U.S. Patent Nos. 5,374,537 and 5,189,056; Tobey, N.A. *et al* (1986) *Am J Physiol* 251:G866-G869; and Orlando, R.C. *et al* (1987) *Gastroenterology* 93:352-361 primarily use acid, acid-pepsin or N-acetylcysteine, a disulfide bond breaking molecule, to mimic the conditions existing *in vivo*.

Further embodiments of the above-described methods therefore include methods wherein the noxious substance may be N-acetylcysteine, acid, acid-pepsin or gastrointestinal fluid. In particular embodiments the noxious substance is gastric acid, such as HCl or HCl-pepsin. In other embodiments the noxious substance may comprise other acids such as phosphoric or sulfuric acid.

Additional noxious substances include bile salts, bile acids, with and without HCl, nitric acid, acetic acid and other organic acids.

Particular embodiments of the invention may also include where the polypeptide is immobilized on a solid support. A variety of appropriate solid supports are known to persons of skill in the art. Exemplary supports may include beads, such as resin beads, slides, glass and plastic wells, test tubes, and plates. The attachment of the polypeptide to a solid support may facilitate performing the steps of the methods of the present invention, including, for example, contacting the polypeptide with a compound or noxious substance. Attachment to a solid support will also facilitate washing the polypeptide between steps. To a person of skill in the art, it will be apparent that any of the above described methods may comprise washing steps between, for example, the contact of the polypeptide with the compound and contact with a noxious substance. Further, washing after contacting the polypeptide with the noxious substance may also be performed before determining, for example, the degree of fragmentation or binding or stability of the polypeptide. The present invention also encompasses embodiments comprising at least one or multiple washing steps between at least one or more of the steps of the assay methods.

The following examples are meant by way of further description of the invention and are not intended to limit the invention described herein in any way.

EXAMPLES

EXAMPLE 1: Identification of the Protein Governing Paracellular Permeability in Rabbit Esophageal Epithelium

Sections of rabbit esophageal epithelium, mounted in Ussing chambers
5 and stabilized for 45 min, were exposed both lumenally and serosally to calcium-free solution containing EDTA, 3 mM. In the presence of a calcium-free solution there was a progressive decline in R, the decline shown for representative single tissues reaching 40% below the initial R at 1 hr, 60% at 2 hrs and 80% at 4 hrs. This contrasted with a control tissue in normal calcium-containing Ringer solution
10 which showed a small increase in R of 10% over initial R at 1 hr, 12% at 2 hrs, and 12% at 4 hrs. The decline in R was the result of the calcium-free state and was not an effect of EDTA treatment since 3 mM EDTA alone had no effect on R over the described time frame.

When calcium-containing (normal Ringer) solution was restored to the
15 bathing solutions after 1, 2, or 4 hrs of calcium-free exposure, there was a progressive return of R toward initial baseline values, with complete reversibility observed for tissue exposed to calcium-free solution for 1 hr and 2 hr exposures and an 80% recovery in R for tissue exposed to calcium-free for 4 hrs within 1 hr of restoring calcium to the baths. These results established that the protein(s)
20 regulating paracellular permeability in the rabbit esophageal epithelium is calcium-dependent.

One protein known to be calcium-sensitive and to act as a bridge to seal off the intercellular space is the zonula adherens junctional protein, Uvomorulin, also known as E-cadherin (and also known as L-CAM, CAM 120/80, Arc-1).
25 Gumbiner, B.M *et al* (1986) *J Cell Biol* 102:457-468.

E-cadherin is known to be insensitive to degradation by trypsin in the presence of calcium but sensitive to degradation in the absence of calcium (Takeichi, M. (1977) *J Cell Biol* 75:464-474). The junctional adhesive protein in esophageal epithelium was shown to be trypsin-sensitive in the absence, but not in
30 the presence, of calcium, which further supports the conclusion that this adhesive

protein is E-cadherin. Sections of rabbit esophageal epithelium were mounted in Ussing chambers and exposed luminally to trypsin, 0.01%, in the presence or absence of a calcium-free bathing solution. There was no significant change in R for tissues exposed to trypsin in the calcium-containing normal Ringer solution over a 2 hr period compared to tissues in calcium-containing normal Ringer not exposed to trypsin (R of $117 \pm 8\%$ vs $107 \pm 8\%$, respectively, $n=4$, $p>0.05$). However, tissues exposed to calcium-free solution 3 mM EDTA both luminally and serosally with or without trypsin showed the expected decline in R over time, as evidenced by declines to $62 \pm 7\%$ and $74 \pm 5\%$, respectively. Upon restoration of calcium to the baths, junctional resealing as evidenced by recovery in R, was shown to be inhibited at 1 hr in tissues exposed to trypsin compared to those unexposed to trypsin ($62 \pm 8\%$ vs $82 \pm 6\%$, respectively, $n=4$, $p<0.05$). This lack of recovery in R in tissues exposed to calcium-free solution suggests that trypsin degraded the junction adhesive protein, while the lack of trypsin's effect on R in calcium-containing solution suggests that the junctional protein is resistant to trypsin under these conditions.

Further, similar to the glycoprotein E-cadherin, whose sugar side chains are known to play little or no role in its ability to provide cell-to-cell adhesion (Takeichi, M. (1988) *Development* 102:639-655), the junctional protein that regulates paracellular permeability in esophageal epithelium was shown to be relatively insensitive to glycosidases. This was evident by the fact that luminal treatment of esophageal epithelium in Ussing Chambers with glycosidases had little ($\leq 20\%$ decrease) or no (decrease approximately 0%) effect on junctional permeability as reflected by change in R.

E-cadherin contains cysteine residues with disulfide bridges in its extracellular domain (Takeichi, M. (1988) *Development* 102:639-655). The junctional protein regulating paracellular permeability in esophageal epithelium is also highly sensitive to disulfide-bond breaking compounds such as N-acetylcysteine. U.S. Pat. No. 5,374,537; 5,189,056. Further, like E-cadherin, which is known to be heat labile, the junctional protein regulating paracellular permeability in esophageal epithelium is heat-sensitive, *i.e.* heating the luminal

bath resulted in a temperature-sensitive decline in R (Tobey, N.A. *et al* (1999) *Am J Physiol* 276:G1322-G1330).

EXAMPLE 2: Junctional Resealing In Esophageal Epithelium is Inhibited by Exposure to Mab to the Extracellular Domain Of E-Cadherin

5 Sections of rabbit esophageal epithelium prepared as in Example 1 were exposed luminally and serosally to a calcium-free solution containing 3 mM EDTA to open the junctions as shown by the progressive decline in R to $60 \pm 4\%$ and $65 \pm 4\%$ of initial R for experimental and control tissues, respectively, by 2 hrs ($n = 8$ for each group, $p > 0.05$). Tissues were then treated luminally for 20 min
10 with either 10 $\mu\text{g/ml}$ of a monoclonal antibody (Mab) to Uvomorulin/E-cadherin (Sigma Chemical, St. Louis, MO) or, as control, to 10 $\mu\text{g/ml}$ of nonspecific immune serum globulin (IgG). Following exposure to Mab or IgG, a calcium-containing (normal Ringer) solution was restored to the bathing solutions and the recovery of R monitored over time. Tissues treated with the Mab to
15 Uvomorulin/E-cadherin showed significant inhibition in recovery of R toward baseline compared to that of tissues treated with IgG; recovery being $70 \pm 5\%$ for Mab vs $86 \pm 6\%$ for IgG by 1 hr after calcium restoration and $73 \pm 4\%$ for Mab vs $88 \pm 4\%$ for IgG by 2 hrs ($p < 0.05$ for each comparison). This indicated that the resealing of the junctions in stratified squamous epithelium is dependent upon the
20 adhesive function of Uvomorulin/E-cadherin.

 The intercellular junctions of stratified squamous epithelium can also be opened by exposure to hypertonic saline. Junctional opening was indicated by a drop in R. Treatment with the same Mab to Uvomorulin/E-cadherin as above also blocked the resealing of the junctions, *i.e.* return of R to baseline, upon
25 reestablishing the isoosmolality of the bathing solution.

 An antibody to the *intracellular* domain of a cadherin (N-cadherin) was also similarly tested and found to have no ability to block junctional resealing, *i.e.* tissues exposed to Mab to the intracellular domain of N-cadherin recovered to $104 \pm 5\%$, using IgG recovery as 100% as control ($n=3$, $p>0.05$). This supports

the specificity for inhibition of junctional resealing by Mab to the *extracellular* domain of Uvomorulin/E-cadherin.

EXAMPLE 3: Junctional Resealing in Esophageal Epithelium is not Inhibited by Mabs to Occludin or Claudin-1

5 Sections of rabbit esophageal epithelium prepared as in Example 1 were exposed luminally and serosally to a calcium-free solution containing 3 mM EDTA to open the junctions as shown by the progressive decline in R over 2 hrs to approximately 40% of initial R. Tissues were then treated luminally for 20 min with either 10 µg/ml of a monoclonal antibody (Mab) to Occludin or a polyclonal
10 antibody to Claudin-1 (both from Zymed, San Francisco, CA), or to nonspecific immune serum globulin (IgG), as control. Following the restoration of calcium-containing (normal Ringer) solution to the bathing solutions, the recovery of R toward baseline was monitored. Unlike the prior inhibition noted with Mab to Uvomorulin/E-cadherin, R had increased similarly by 2 hrs after calcium
15 replacement for tissues exposed to antibodies to occludin or claudin-1 compared with the IgG controls. For example, at 2 hrs post-calcium restoration, using IgG recovery as 100% as control, tissues exposed to Mab for occludin recovered to 96%±6%, and to polyclonal antibodies to claudin-1 recovered to 108%±8% (n=3 per group, p> 0.05 compared to control IgG for each group). This demonstrated
20 that junctional resealing in esophageal epithelium was independent of the adhesive function of occludin or claudin-1, junctional proteins which are not members of the cadherin superfamily of proteins.

EXAMPLE 4: Junctional Resealing in Esophageal Epithelium Inhibited by Mab to Desmogleins 1 and 2 and Desmocollin 3

25 Sections of rabbit esophageal epithelium prepared as in Example 1 were exposed luminally and serosally to calcium-free solution containing 3 mM EDTA to open the junctions as shown by the progressive decline in R over 2 hrs to approximately 40% of initial R. Tissues were then treated luminally for 20 min with either 10 µg/ml of a Mab to desmoglein 1 and 2 (Research Diagnostics,
30 Flanders, NJ), desmocollin 3 (Research Diagnostics, Flanders, NJ),

Uvomorulin/E-cadherin (Sigma Chemical, St. Louis, MO), or nonspecific immune serum globulin (IgG) as control. Following restoration of calcium-containing (normal Ringer) solution, the Mab for desmoglein, desmocollin and Uvomorulin/E-cadherin were observed to significantly inhibit return of R toward initial values compared to controls exposed to IgG. For example, when expressed as a percent recovery in relation to IgG (IgG recovery normalized to 100%) at 2 hrs, Uvomorulin Mab was $83 \pm 6\%$, desmocollin Mab was $83 \pm 0.5\%$ and desmoglein 1 and 2 Mab was $90 \pm 8\%$ ($n=4-6/\text{group}$, $p<0.05$ for each group compared to the IgG control). This indicated that junctional resealing in esophageal epithelium can also be inhibited by Mab to desmoglein 1 and 2, and desmocollin 3, which are members of the cadherin superfamily of proteins with known sequence homology to Uvomorulin/E-cadherin.

The ability of desmosomal antibodies for desmoglein 1 and 2 or desmocollin 3 to block resealing similar to that of Mab to Uvomorulin/E-cadherin is likely due to homology between the proteins desmoglein 1 and 2, desmocollin 3 and Uvomorulin/E-cadherin. Desmosomes are structures that provide tensile strength to the epithelium but do not regulate permeability through the paracellular pathway, and thus the mechanism of action is not by Mab binding to desmogleins 1 or 2, or desmocollin 3. (Gumbiner, B.M. (1996) *Cell* 84:345-357, Swami, S. *et al* (1995) *Am J Gastroenterol* 90:1808-1813, Dobson, H. *et al* (1994) *Gut* 35:1343-1347).

EXAMPLE 5: Method for Measuring Mab Competition with Mab to E-cadherin for Binding to Extracellular Domain of E-cadherin

A commercial immunoassay test kit (Human E-cadherin EIA kit, Zymed Laboratories, San Francisco, CA) that contains an antigen (Uvomorulin/E-cadherin), and a Mab to the extracellular domain of Uvomorulin/E-cadherin was used to demonstrate that Mab to desmocollin 3 can bind to the extracellular domain of Uvomorulin/E-cadherin.

The kit enables the antigen to be exposed to agents or antibodies prior to exposure to the standard Mab to the extracellular domain of Uvomorulin/E-cadherin. The ability of the agent or antibody to block the binding

of the Mab to the extracellular domain of Uvomorulin/E-cadherin can then be used to determine if competition for binding sites on the antigen has occurred. Comparative binding is based on the quantity of binding observed when the Mab to Uvomorulin/E-cadherin is placed in contact with antigen in the absence of prior exposure to test agent or antibody. The experimental control for test agents is vehicle (normal Ringer solution) and for monoclonal or polyclonal antibodies is an equivalent amount of nonspecific immune serum globulin (IgG). The quantity of binding of the Mab to Uvomorulin/E-cadherin to antigen in the absence of test agent or test antibody is labeled as control and whose value is normalized to 100%.

Antigen which was first exposed to a similar quantity of a Mab to desmocollin 3 (3 μ g/100 μ L) (Research Diagnostics, Flanders, NJ) prior to exposure of the Mab to Uvomorulin/E-cadherin demonstrated significantly reduced binding of Uvomorulin/E-cadherin Mab (64 \pm 5.8% compared to 3 μ g/100 μ L of IgG as control, $p < 0.05$, $n = 6$). This indicated that the Mab to desmocollin 3 competes effectively for the same binding sites as the Mab to the extracellular domain of Uvomorulin/E-cadherin.

Without being bound to any particular theory, it appears, in effect, that the Mab to desmoglein 1 and 2, and desmocollin 3 exert their effect on resealing by binding to the extracellular domain of Uvomorulin/E-cadherin. This conclusion is further supported by the fact that the Mab to desmoglein 1 and 2, and desmocollin 3 cross react on SDS gel electrophoresis with one or more of the same protein bands as the Mab to Uvomorulin/E-cadherin.

EXAMPLE 6: SITS Provides Protection Against Noxious Substances

Uvomorulin/E-cadherin was identified by SDS gel electrophoresis in homogenates of esophageal epithelium. These findings are supported by reports from others of the presence of Uvomorulin/E-cadherin on immunostaining, and on SDS gel electrophoresis in human esophageal epithelium (Swami, S. *et al* (1995) *Am J Gastroenterol* 90:1808-1813).

When epithelia were treated with acid-pepsin or N-acetylcysteine and then homogenized, bands which stain with the Mab to E-cadherin are visible at 50kD and 80kD. The band at approximately 80 kD should correspond to the extracellular domain of E-cadherin, as identified elsewhere, after treatment of E-cadherin with trypsin in the absence of calcium, an approximately 80kD band is identified. Gumbiner, B.M. *et al.* (1986) *J Cell Biol* 102:457-468.

SITS was selected to investigate the mechanism of protection because of its commercial availability, high level of protection against acid injury to esophageal epithelium, and lack of reversibility of its protective effect even with repeated washes of the tissue prior to acid exposure. When epithelia were treated first with SITS, and then contacted with acid-pepsin or N-acetylcysteine, the two bands at approximately 50 kD were not seen, suggesting that the cytoprotective agent SITS prevents the breakdown or fragmentation of the extracellular domain in the presence of the noxious substances acid-pepsin and N-acetylcysteine.

Protection of Uvomorulin/E-cadherin against damage by acid or N-acetylcysteine was shown to be direct, *i.e.*, by binding of the compound to the protein itself (rather than indirect by the binding of the compound to other proteins providing a shield against the access of acid or N-acetylcysteine to the critical junctional protein). Sections of rabbit esophageal epithelium were mounted in Ussing chambers and exposed luminally to either SITS, 4 mM, or vehicle (Ringer) as control for 30 min. Following exposures, both bathing solutions were made calcium-free solutions (3 mM EDTA and 4 mM SITS, or 3 mM EDTA without SITS (control)) to open the junctions as evidenced by the decline in R. In the presence of calcium-free solution with or without 4 mM SITS, R over a 2 hr period declined similarly for the two groups to $68 \pm 3\%$ or $67 \pm 3\%$, respectively. However, recovery of R following restoration of calcium-containing bathing solutions (0 mM SITS) showed that the recovery of R of tissues previously exposed to SITS was significantly reduced over that of the unexposed tissue (SITS treated recovery of $75 \pm 3\%$ vs untreated controls of $90 \pm 5\%$, $n=3$, $p<0.05$). This showed that luminal SITS inhibits junctional

resealing, a function of the adhesive protein E-cadherin, and this interference is likely through its ability to bind to the extracellular domain of E-cadherin.

EXAMPLE 7: Method for Determining the Ability of Compounds to Bind to the Extracellular Domain of Uvomorulin/E-Cadherin

5 The Human E-cadherin EIA kit (Zymed Laboratories, San Francisco, CA) was used as in the method described in Example 5 to determine binding of test compounds, SITS, SOS, CDDD-1192, CDDD-1193 and 1,3-benzene disulfonic acid (1,3-BDSA) to the approximately 80 kD extracellular domain of Uvomorulin/E-Cadherin provided in the E-cadherin EIA kit. This was done by
10 adding the extracellular domain of human Uvomorulin/E-Cadherin to a solution with varying concentrations of the compounds in the nanomolar to millimolar range or with vehicle (control, normal Ringer solution) for 30 min. Following incubation for 2 hrs, the solution is added to a solid phase microtiter plate, provided in the EIA kit, to which a mouse monoclonal anti-human Uvomorulin/E-
15 cadherin antibody (Zymed Laboratories, San Francisco, CA) is bound. This permits antigen with epitopes unoccupied by test compound to bind to the plate. After washing the plate with Tris buffer containing 0.1% Tween-20 (Promega, Madison, Wisconsin) and 5 mM calcium chloride, the second mouse monoclonal anti-human Uvomorulin/E-cadherin antibody labeled with peroxidase is used to
20 tag the bound antigen. After an additional incubation for 1 hr and washing with Tris buffer, the peroxidase is detected with substrate (hydrogen peroxide and tetramethyl-benzidine in buffered solution, as included in the EIA test kit) by incubating for 15 min at room temperature and stopping of the reaction with 1N H₂O₄. This results in color development with intensities proportional to the
25 amount of Uvomorulin/E-cadherin present in the samples. The amount of Uvomorulin/E-cadherin is quantitated by measuring absorbance at 450 nm within an hour following development using an EIA plate reader (Vmax Plate Reader, Molecular Devices, Sunnyvale, CA). The degree of binding of a compound to the extracellular domain of Uvomorulin/E-cadherin is determined by the reduction in
30 antigen detection in the plate exposed to test compound compared to that for the control plate (100%) where no compound was present (Table 2).

Table 2. The ability of selected agents to alter binding of a Mab to the extracellular domain of Uvomorulin/E-cadherin to its antigenic sites.

Agent	Concentration	% Binding
Control (vehicle)		100
1,3 BDSA	4 mM	104±3
SITS	40 uM	77±3*
SITS	4 mM	26±3*
SITS	8 mM	20±3*
SOS	4 mM	83±0.3 *
SOS	10 mM	76±0.3 *
CDDD-1192	0.4 uM	103±6
CDDD-1192	4 mM	77±13 *
CDDD-1193	40 nM	110±6
CDDD-1193	40 uM	83±3 *
CDDD-1193	4 mM	38±0.75 *

5

* p <0.05 compared to control; n=3-6 for each experimental group

The experimental results depicted in Table 2 establish the ability of protective doses of SITS, SOS, CDDD-1192 and CDDD-1193, but not non-protective doses of 1,3-BDSA, a compound ineffective against acid injury in the Ussing chamber experiments at a concentration of 4 mM (as shown in U.S. Patent No. 5,189,056 and 5,374,537), to bind to the extracellular domain of E-cadherin. Further, the binding affinity for the epitope identified by the Mab to the extracellular domain for E-cadherin is greatest for CDDD-1193 and SITS at 4 mM concentrations when compared to that of CDDD-1192 or SOS.

15

Esophageal epithelium lumenally pretreated with SITS (even without prior junctional opening by calcium-free solution) is found to inhibit the binding of a Mab to Uvomorulin/E-cadherin to its antigen (Uvomorulin/E-cadherin) when applied to homogenized epithelium run on SDS gel electrophoresis.

5 Additionally, esophageal epithelium lumenally pretreated with SITS in Ussing chambers that were subsequently exposed to acid-pepsin or N-acetylcysteine were resistant to the degradation of Uvomorulin/E-cadherin into component proteins (Example 6). This was demonstrated by showing additional protein bands with Mab staining for Uvomorulin/E-cadherin of homogenized
10 esophageal epithelium on SDS gel electrophoresis for tissues exposed to acid-pepsin or N-acetylcysteine compared to similarly exposed tissues that were pretreated with luminal SITS.

 Taken together, these data support the conclusion that the previously identified lumenally-active cytoprotective compounds inhibit the junctional
15 breakdown (and concomitant increase in paracellular permeability) in esophageal epithelium upon direct damage by acid, acid-pepsin or N-acetylcysteine, by direct binding to the extracellular domain of Uvomorulin/E-cadherin. Without being bound by any particular theory, it appears that the mechanism of action of these compounds is the stabilization of the target protein, *i.e.* Uvomorulin/E-cadherin,
20 which results in protection against acid, acid-pepsin or N-acetylcysteine induced increases in junctional (paracellular) permeability.

 It appears that sulfonates like SITS and sulfate esters, *e.g.* SOS, CDDD-1192 and CDDD-1193, act via the same mechanism. Sucrose octasulfate (previously shown to irreversibly protect against acid-induced injury to
25 esophageal epithelium (U.S. Patent No. 5,374,537 and 5,189,056)) also dose-dependently binds to the extracellular domain of Uvomorulin/E-cadherin.

 A third category of alleged cytoprotective compounds known as inorganic tetrahedral-shaped oxyanions containing compounds such as sulfates, molybdates, chromates, and tungstates did not, like organic sulfonates and sulfate esters,
30 inhibit binding of the Mab to the extracellular domain of Uvomorulin/E-cadherin to its antigen, Uvomorulin/E-cadherin, using a commercially available

immunoassay kit, as described above. This likely reflects the fact that these compounds demonstrate readily reversible protection against acid injury in the Ussing chamber, such that their binding to the protective site is most likely reversible and so ineffective at inhibiting the Mab to Uvomorulin/E-cadherin from binding to its antigen. Thus, compounds like the inorganic tetrahedral-shaped oxyanions described may show cytoprotective activity in rabbit esophageal epithelial tissue models as described in U.S. Pat. Nos. 5,374,537 and 5,189,056, but may not be suitable for use as control cytoprotective agents in the assay methods described herein. Further, the reversibility of binding suggests that these compounds may also not be as effective *in vivo*, where the esophageal tissues are being washed by saliva.

Identification of the isolated extracellular domain of Uvomorulin/E-cadherin as the protective site supports use of this protein and its extracellular domain specifically as a test system for identifying, screening, selecting and designing compounds, chemicals, molecules, antibodies or other substances for protective effects against luminal damaging compounds, *e.g.* HCl, to stratified squamous epithelia.

Modifications of the above described modes for carrying out the invention that are obvious to those of ordinary skill in the chemical, pharmaceutical, or related arts are intended to be within the scope of the following claims.

CLAIMS

1. A method of assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising in order:
- 5 a) contacting a polypeptide with a compound; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted within the extracellular domain of **SEQ ID NO:1**; and
- 10 b) determining whether the compound binds to the polypeptide; wherein binding of the compound to the polypeptide indicates that the compound may protect stratified squamous epithelia from damage by a noxious substance.
2. The method of claim 1, further comprising:
- 15 c) comparing the binding of the compound to the polypeptide with the binding of a cytoprotective agent to the polypeptide; wherein a greater than or equal level of binding by the compound indicates that the compound may protect stratified squamous epithelia from damage by the noxious substance.
3. The method of claim 2, wherein the cytoprotective agent is chosen
- 20 from the group consisting of sulfate esters and sulfonates.
4. The method of claim 3, wherein the cytoprotective agent is chosen from the group consisting of SITS, SOS, CDDD-1192 and CDDD-1193.
5. The method of claim 1, wherein the polypeptide is an extracellular portion of E-cadherin.
- 25 6. The method of claim 5, wherein the extracellular portion comprises at least 5 residues of the extracellular domain of E-cadherin.
7. The method of claim 1, wherein the polypeptide comprises at least 5 residues of a sequence depicted within one of **SEQ ID NOS:6-21**, **SEQ ID NOS:22-25**, or within the extracellular domain of **SEQ ID NO:2**, **SEQ ID NO:3**,
- 30 **SEQ ID NO:4**; or **SEQ ID NO:5**.

8. The method of claim 1, wherein the polypeptide comprises at least 5 residues of a sequence depicted within residues 155-262 of **SEQ ID NO:1**, residues 52-157 of **SEQ ID NO:2**, residues 49-159 of **SEQ ID NO:3**, residues 52-157 of **SEQ ID NO:4**, and residues 136-243 of **SEQ ID NO:5**.

5 9. The method of claim 1, wherein the compound is selected from the group consisting of sulfate esters and sulfonates.

10. The method of claim 1, further comprising, in order:

c) contacting a sample comprising stratified squamous epithelial tissue with the compound;

10 d) contacting the sample with a noxious substance; and

e) determining if the epithelia is damaged.

11. The method of claim 10, wherein the epithelia is selected from the group of epithelia consisting of esophageal, oral cavity, cheek, pharynx, larynx and vocal cord tissue.

15 12. The method of claim 10, wherein the epithelia comprises rabbit esophageal epithelia.

13. The method of claim 10, wherein the noxious substance is selected from the group consisting of gastric acid, HCl, N-acetylcysteine, pepsin, acid-pepsin, and gastrointestinal fluid.

20 14. The method of claim 13, wherein the noxious substance is HCl.

15. The method of claim 13, wherein the noxious substance is HCl-pepsin.

16. The method of claim 1, wherein the polypeptide further comprises a solid support.

17. The method of claim 16, wherein the solid support is chosen from the group consisting of resin beads, glass slides, plastic slides, glass test tubes, plastic test tubes, glass wells, plastic wells, glass plates and plastic plates.

18. A method of assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising in order:

30 a) contacting a polypeptide with a labeled cytoprotective agent; wherein the polypeptide is selected from the group consisting of an extracellular portion of

a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted within the extracellular domain of **SEQ ID NO:1**;

b) contacting the polypeptide with a compound; and

5 c) comparing binding of the agent to the polypeptide contacted with the compound to a control conducted where the polypeptide is contacted with the agent and is not contacted with the compound;

wherein a decrease in binding of the agent to the polypeptide contacted with the compound compared to the control indicates that the compound may protect stratified squamous epithelia against damage by a noxious substance.

10 19. The method of claim 18, wherein the agent is labeled with a radioactive isotope, fluorescent label or electron dense tracer.

20. The method of claim 19, wherein the electron dense tracer is selected from the group consisting of horseradish peroxidase, glucose oxidase, and alkaline phosphatase.

15 21. A method for identifying a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising, in order:

a) contacting a polypeptide with a compound; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted
20 within the extracellular domain of **SEQ ID NO:1**;

b) contacting the polypeptide with a monoclonal antibody to the polypeptide; and

c) comparing the amount of antibody binding to the polypeptide contacted with the compound to the amount of antibody binding to a control;

25 wherein a decrease in the amount of antibody binding to the polypeptide which has been contacted with the compound compared to the control indicates that the compound may protect stratified squamous epithelia from damage by a noxious substance.

30 22. The method of claim 21, wherein the control comprises contacting the polypeptide with the monoclonal antibody or wherein the control comprises

contacting the polypeptide with a cytoprotective agent and then contacting the polypeptide with the monoclonal antibody.

23. A method of assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising in order:

- 5 a) contacting a polypeptide with a compound; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted with in the extracellular domain of SEQ ID NO:1;
- b) determining the stability of the polypeptide; and
- 10 c) comparing the stability of the polypeptide contacted with the compound to the stability of a control;

wherein a greater stability for the polypeptide contacted with the compound compared to the control indicates that the compound may protect stratified squamous epithelia from damage by a noxious substance.

- 15 24. The method of claim 23, wherein the control comprises where the polypeptide is not contacted with the compound or where the polypeptide is contacted with a cytoprotective agent and is not contacted with the compound.

25. The method of claim 23, wherein stability is determined using a technique selected from the group consisting of calorimetry; titration of chemical
- 20 denaturation and titration of thermal denaturation.

26. A method of assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising in order:

- 25 a) contacting a polypeptide with the compound; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted within the extracellular domain of SEQ ID NO:1;
- b) contacting the polypeptide with a noxious substance;
- 30 c) determining the stability of the polypeptide; and

d) comparing the stability of the polypeptide contacted with the compound and the noxious substance to the stability of a control;

wherein if the stability of the polypeptide contacted with the compound and noxious substance is greater than that of the control, the compound may protect stratified squamous epithelium against damage by the noxious substance.

27. The method of claim 26, wherein the control comprises where the polypeptide is not contacted with the compound but is contacted with the noxious substance or where the polypeptide is contacted with a cytoprotective agent and then contacted with the noxious substance.

28. The method of claim 26, wherein stability is determined using a technique selected from the group consisting of calorimetry; titration of chemical denaturation and titration of chemical denaturation.

29. A method of assaying for a compound which may protect stratified squamous epithelia from damage by a noxious substance, comprising in order:

a) contacting a polypeptide with the compound; wherein the polypeptide is selected from the group consisting of an extracellular portion of a cadherin or a peptide sequence having at least 13% sequence identity to a sequence depicted within the extracellular domain of **SEQ ID NO:1**;

b) contacting the polypeptide with a noxious substance;

c) determining the amount of fragmentation of the polypeptide; and

d) comparing the amount of fragmentation of the polypeptide to a control;

wherein a decrease in the amount of fragmentation of the polypeptide contacted with the compound compared to the control indicates that the compound may protect stratified squamous epithelia from damage by a noxious substance.

30. The method of claim 29, wherein the control comprises where the polypeptide is not contacted with the compound but is contacted with the noxious substance or where the polypeptide is contacted with a cytoprotective agent and then contacted with the noxious substance.

31. The method of claim 29, wherein fragmentation is determined using a technique from the group consisting of SDS gel electrophoresis, mass spectrometry, HPLC, or amino acid analysis.

5 32. The method of claim 29, wherein the technique is SDS gel electrophoresis.

SEQUENCE LISTING

<110> The Administrators of the Tulane Educational Fund
 Tobey, Nelia A.
 Orlando, Roy C.

<120> Assay Methods for Identifying Compounds
 Which May Protect Stratified Squamous Epithelium Against
 Damage By Noxious Substances

<130> 484482000240

<140> To Be Assigned
 <141> Herewith

<150> 09/626,196
 <151> 2000-07-28

<160> 25

<170> FastSEQ for Windows Version 4.0

<210> 1
 <211> 882
 <212> PRT
 <213> Homo Sapien

<220>
 <221> REPEAT
 <222> (155)...(262)
 <223> Extracellular subdomain 1

<221> REPEAT
 <222> (263)...(375)
 <223> Extracellular subdomain 2

<221> REPEAT
 <222> (376)...(486)
 <223> Extracellular subdomain 3

<221> REPEAT
 <222> (487)...(593)
 <223> Extracellular subdomain 4

<221> REPEAT
 <222> (594)...(697)
 <223> Extracellular subdomain 5

<221> DOMAIN
 <222> (155)...(707)
 <223> Extracellular domain

<400> 1
 Met Gly Pro Trp Ser Arg Ser Leu Ser Ala Leu Leu Leu Leu Gln
 1 5 10 15
 Val Ser Ser Trp Leu Cys Gln Glu Pro Glu Pro Cys His Pro Gly Phe
 20 25 30
 Asp Ala Glu Ser Tyr Thr Phe Thr Val Pro Arg Arg His Leu Glu Arg
 35 40 45

Gly Arg Val Leu Gly Arg Val Asn Phe Glu Asp Cys Thr Gly Arg Gln
 50 55 60
 Arg Thr Ala Tyr Phe Ser Leu Asp Thr Arg Phe Lys Val Gly Thr Asp
 65 70 75 80
 Gly Val Ile Thr Val Lys Arg Pro Leu Arg Phe His Asn Pro Gln Ile
 85 90 95
 His Phe Leu Val Tyr Ala Trp Asp Ser Thr Tyr Arg Lys Phe Ser Thr
 100 105 110
 Lys Val Thr Leu Asn Thr Val Gly His His His Arg Pro Pro Pro His
 115 120 125
 Gln Ala Ser Val Ser Gly Ile Gln Ala Glu Leu Leu Thr Phe Pro Asn
 130 135 140
 Ser Ser Pro Gly Leu Arg Arg Gln Lys Arg Asp Trp Val Ile Pro Pro
 145 150 155 160
 Ile Ser Cys Pro Glu Asn Glu Lys Gly Pro Phe Pro Lys Asn Leu Val
 165 170 175
 Gln Ile Lys Ser Asn Lys Asp Lys Glu Gly Lys Val Phe Tyr Ser Ile
 180 185 190
 Thr Gly Gln Gly Ala Asp Thr Pro Val Gly Val Phe Ile Ile Glu
 195 200 205
 Arg Glu Thr Gly Trp Leu Lys Val Thr Glu Pro Leu Asp Arg Glu Arg
 210 215 220
 Ile Ala Thr Tyr Thr Leu Phe Ser His Ala Val Ser Ser Asn Gly Asn
 225 230 235 240
 Ala Val Glu Asp Pro Met Glu Ile Leu Ile Thr Val Thr Asp Gln Asn
 245 250 255
 Asp Asn Lys Pro Glu Phe Thr Gln Glu Val Phe Lys Gly Ser Val Met
 260 265 270
 Glu Gly Ala Leu Pro Gly Thr Ser Val Met Glu Val Thr Ala Thr Asp
 275 280 285
 Ala Asp Asp Asp Val Asn Thr Tyr Asn Ala Ala Ile Ala Tyr Thr Ile
 290 295 300
 Leu Ser Gln Asp Pro Glu Leu Pro Asp Lys Asn Met Phe Thr Ile Asn
 305 310 315 320
 Arg Asn Thr Gly Val Ile Ser Val Val Thr Thr Gly Leu Asp Arg Glu
 325 330 335
 Ser Phe Pro Thr Tyr Thr Leu Val Val Gln Ala Ala Asp Leu Gln Gly
 340 345 350
 Glu Gly Leu Ser Thr Thr Ala Thr Ala Val Ile Thr Val Thr Asp Thr
 355 360 365
 Asn Asp Asn Pro Pro Ile Phe Asn Pro Thr Thr Tyr Lys Gly Gln Val
 370 375 380
 Pro Glu Asn Glu Ala Asn Val Val Ile Thr Thr Leu Lys Val Thr Asp
 385 390 395 400
 Ala Asp Ala Pro Asn Thr Pro Ala Trp Glu Ala Val Tyr Thr Ile Leu
 405 410 415
 Asn Asp Asp Gly Gly Gln Phe Val Val Thr Thr Asn Pro Val Asn Asn
 420 425 430
 Asp Gly Ile Leu Lys Thr Ala Lys Gly Leu Asp Phe Glu Ala Lys Gln
 435 440 445
 Gln Tyr Ile Leu His Val Ala Val Thr Asn Val Val Pro Phe Glu Val
 450 455 460
 Ser Leu Thr Thr Ser Thr Ala Thr Val Thr Val Asp Val Leu Asp Val
 465 470 475 480
 Asn Glu Ala Pro Ile Phe Val Pro Pro Glu Lys Arg Val Glu Val Ser
 485 490 495
 Glu Asp Phe Gly Val Gly Gln Glu Ile Thr Ser Tyr Thr Ala Gln Glu
 500 505 510
 Pro Asp Thr Phe Met Glu Gln Lys Ile Thr Tyr Arg Ile Trp Arg Asp
 515 520 525
 Thr Ala Asn Trp Leu Glu Ile Asn Pro Asp Thr Gly Ala Ile Ser Thr

530	Arg	Ala	Glu	Leu	Asp	535	Arg	Glu	Asp	Phe	Glu	His	Val	Lys	Asn	Ser	Thr
545	Tyr	Thr	Ala	Leu	Ile	550	Ile	Ala	Thr	Asp	Asn	Gly	Ser	Pro	Val	Ala	Thr
				565						570						575	
	Gly	Thr	Gly	Thr	Leu		Leu	Leu	Ile	Leu	Ser	Asp	Val	Asn	Asp	Asn	Ala
				580						585						590	
	Pro	Ile	Pro	Glu	Pro		Arg	Thr	Ile	Phe	Phe	Cys	Glu	Arg	Asn	Pro	Lys
				595					600					605			
	Pro	Gln	Val	Ile	Asn		Ile	Ile	Asp	Ala	Asp	Leu	Pro	Pro	Asn	Thr	Ser
				610				615						620			
	Pro	Phe	Thr	Ala	Glu		Leu	Thr	His	Gly	Ala	Ser	Ala	Asn	Trp	Thr	Ile
	625						630						635				640
	Gln	Tyr	Asn	Asp	Pro		Thr	Gln	Glu	Ser	Ile	Ile	Leu	Lys	Pro	Lys	Met
				645							650						655
	Ala	Leu	Glu	Val	Gly		Asp	Tyr	Lys	Ile	Asn	Leu	Lys	Leu	Met	Asp	Asn
				660						665						670	
	Gln	Asn	Lys	Asp	Gln		Val	Thr	Thr	Leu	Glu	Val	Ser	Val	Cys	Asp	Cys
				675					680							685	
	Glu	Gly	Ala	Ala	Gly		Val	Cys	Arg	Lys	Ala	Gln	Pro	Val	Glu	Ala	Gly
				690				695					700				
	Leu	Gln	Ile	Pro	Ala		Ile	Leu	Gly	Ile	Leu	Gly	Gly	Ile	Leu	Ala	Leu
	705						710					715					720
	Leu	Ile	Leu	Ile	Leu		Leu	Leu	Leu	Leu	Phe	Leu	Arg	Arg	Arg	Ala	Val
				725						730							735
	Val	Lys	Glu	Pro	Leu		Leu	Pro	Pro	Glu	Asp	Asp	Thr	Arg	Asp	Asn	Val
				740						745						750	
	Tyr	Tyr	Tyr	Asp	Glu		Glu	Gly	Gly	Gly	Glu	Glu	Asp	Gln	Asp	Phe	Asp
				755					760						765		
	Leu	Ser	Gln	Leu	His		Arg	Gly	Leu	Asp	Ala	Arg	Pro	Glu	Val	Thr	Arg
				770				775					780				
	Asn	Asp	Val	Ala	Pro		Thr	Leu	Met	Ser	Val	Pro	Arg	Tyr	Leu	Pro	Arg
	785						790						795				800
	Pro	Ala	Asn	Pro	Asp		Glu	Ile	Gly	Asn	Phe	Ile	Asp	Glu	Asn	Leu	Lys
				805						810						815	
	Ala	Ala	Asp	Thr	Asp		Pro	Thr	Ala	Pro	Pro	Tyr	Asp	Ser	Leu	Leu	Val
				820						825					830		
	Phe	Asp	Tyr	Glu	Gly		Ser	Gly	Ser	Glu	Ala	Ala	Ser	Leu	Ser	Ser	Leu
				835					840					845			
	Asn	Ser	Ser	Glu	Ser		Asp	Lys	Asp	Gln	Asp	Tyr	Asp	Tyr	Leu	Asn	Glu
				850				855					860				
	Trp	Gly	Asn	Arg	Phe		Lys	Lys	Leu	Ala	Asp	Met	Tyr	Gly	Gly	Gly	Glu
	865						870					875					880
	Asp	Asp															

<210> 2

<211> 1049

<212> PRT

<213> Homo Sapien

<220>

<221> DOMAIN

<222> (50)...(548)

<223> Extracellular domain

<400> 2

Met Asp Trp Ser Phe Phe Arg Val Val Ala Val Leu Phe Ile Phe Leu

1

5

10

15

Val Val Val Glu Val Asn Ser Glu Phe Arg Ile Gln Val Arg Asp Tyr

20

25

30

Asn Thr Lys Asn Gly Thr Ile Lys Trp His Ser Ile Arg Arg Gln Lys
 35 40 45
 Arg Glu Trp Ile Lys Phe Ala Ala Ala Cys Arg Glu Gly Glu Asp Asn
 50 55 60
 Ser Lys Arg Asn Pro Ile Ala Lys Ile His Ser Asp Cys Ala Ala Asn
 65 70 75 80
 Gln Gln Val Thr Tyr Arg Ile Ser Gly Val Gly Ile Asp Gln Pro Pro
 85 90 95
 Tyr Gly Ile Phe Val Ile Asn Gln Lys Thr Gly Glu Ile Asn Ile Thr
 100 105 110
 Ser Ile Val Asp Arg Glu Val Thr Pro Phe Phe Ile Ile Tyr Cys Arg
 115 120 125
 Ala Leu Asn Ser Met Gly Gln Asp Leu Glu Arg Pro Leu Glu Leu Arg
 130 135 140
 Val Arg Val Leu Asp Ile Asn Asp Asn Pro Pro Val Phe Ser Met Ala
 145 150 155 160
 Thr Phe Ala Gly Gln Ile Glu Glu Asn Ser Asn Ala Asn Thr Leu Val
 165 170 175
 Met Ile Leu Asn Ala Thr Asp Ala Asp Glu Pro Asn Asn Leu Asn Ser
 180 185 190
 Lys Ile Ala Phe Lys Ile Ile Arg Gln Glu Pro Ser Asp Ser Pro Met
 195 200 205
 Phe Ile Ile Asn Arg Asn Thr Gly Glu Ile Arg Thr Met Asn Asn Phe
 210 215 220
 Leu Asp Arg Glu Gln Tyr Gly Gln Tyr Ala Leu Ala Val Arg Gly Ser
 225 230 235 240
 Asp Arg Asp Gly Gly Ala Asp Gly Met Ser Ala Glu Cys Glu Cys Asn
 245 250 255
 Ile Lys Ile Leu Asp Val Asn Asp Asn Ile Pro Tyr Met Glu Gln Ser
 260 265 270
 Ser Tyr Thr Ile Glu Ile Gln Glu Asn Thr Leu Asn Ser Asn Leu Leu
 275 280 285
 Glu Ile Arg Val Ile Asp Leu Asp Glu Glu Phe Ser Ala Asn Trp Met
 290 295 300
 Ala Val Ile Phe Phe Ile Ser Gly Asn Glu Gly Asn Trp Phe Glu Ile
 305 310 315 320
 Glu Met Asn Glu Arg Thr Asn Val Gly Ile Leu Lys Val Val Lys Pro
 325 330 335
 Leu Asp Tyr Glu Ala Met Gln Ser Leu Gln Leu Ser Ile Gly Val Arg
 340 345 350
 Asn Lys Ala Glu Phe His His Ser Ile Met Ser Gln Tyr Lys Leu Lys
 355 360 365
 Ala Ser Ala Ile Ser Val Thr Val Leu Asn Val Ile Glu Gly Pro Val
 370 375 380
 Phe Arg Pro Gly Ser Lys Thr Tyr Val Val Thr Gly Asn Met Gly Ser
 385 390 395 400
 Asn Asp Lys Val Gly Asp Phe Val Ala Thr Asp Leu Asp Thr Gly Arg
 405 410 415
 Pro Ser Thr Thr Val Arg Tyr Val Met Gly Asn Asn Pro Ala Asp Leu
 420 425 430
 Leu Ala Val Asp Ser Arg Thr Gly Lys Leu Thr Leu Lys Asn Lys Val
 435 440 445
 Thr Lys Glu Gln Tyr Asn Met Leu Gly Gly Lys Tyr Gln Gly Thr Ile
 450 455 460
 Leu Ser Ile Asp Asp Asn Leu Gln Arg Thr Cys Thr Gly Thr Ile Asn
 465 470 475 480
 Ile Asn Ile Gln Ser Phe Gly Asn Asp Asp Arg Thr Asn Thr Glu Pro
 485 490 495
 Asn Thr Lys Ile Thr Thr Asn Thr Gly Arg Gln Glu Ser Thr Ser Ser
 500 505 510
 Thr Asn Tyr Asp Thr Ser Thr Thr Ser Thr Asp Ser Ser Gln Val Tyr

515					520					525					
Ser	Ser	Glu	Pro	Gly	Asn	Gly	Ala	Lys	Asp	Leu	Leu	Ser	Asp	Asn	Val
	530					535					540				
His	Phe	Gly	Pro	Ala	Gly	Ile	Gly	Leu	Leu	Ile	Met	Gly	Phe	Leu	Val
545					550					555					560
Leu	Gly	Leu	Val	Pro	Phe	Leu	Met	Ile	Cys	Asp	Cys	Gly	Gly	Ala	
				565					570					575	
Pro	Arg	Ser	Ala	Gly	Phe	Glu	Pro	Val	Pro	Glu	Cys	Ser	Asp	Gly	
			580				585					590			
Ala	Ile	His	Ser	Trp	Ala	Val	Glu	Gly	Pro	Gln	Pro	Glu	Pro	Arg	Asp
		595				600					605				
Ile	Thr	Thr	Val	Ile	Pro	Gln	Ile	Pro	Pro	Asp	Asn	Ala	Asn	Ile	Ile
	610					615					620				
Glu	Cys	Ile	Asp	Asn	Ser	Gly	Val	Tyr	Thr	Asn	Glu	Tyr	Gly	Gly	Arg
625					630					635					640
Glu	Met	Gln	Asp	Leu	Gly	Gly	Gly	Glu	Arg	Met	Thr	Gly	Phe	Glu	Leu
				645					650					655	
Thr	Glu	Gly	Val	Lys	Thr	Ser	Gly	Met	Pro	Glu	Ile	Cys	Gln	Glu	Tyr
			660					665					670		
Ser	Gly	Thr	Leu	Arg	Arg	Asn	Ser	Met	Arg	Glu	Cys	Arg	Glu	Gly	Gly
		675				680					685				
Leu	Asn	Met	Asn	Phe	Met	Glu	Ser	Tyr	Phe	Cys	Gln	Lys	Ala	Tyr	Ala
					695					700					
Tyr	Ala	Asp	Glu	Asp	Glu	Gly	Arg	Pro	Ser	Asn	Asp	Cys	Leu	Leu	Ile
705					710					715					720
Tyr	Asp	Ile	Glu	Gly	Val	Gly	Ser	Pro	Ala	Gly	Ser	Val	Gly	Cys	Cys
				725					730					735	
Ser	Phe	Ile	Gly	Glu	Asp	Leu	Asp	Asp	Ser	Phe	Leu	Asp	Thr	Leu	Gly
			740					745					750		
Pro	Lys	Phe	Lys	Lys	Leu	Ala	Asp	Ile	Ser	Leu	Gly	Lys	Glu	Ser	Tyr
		755				760					765				
Pro	Asp	Leu	Asp	Pro	Ser	Trp	Pro	Pro	Gln	Ser	Thr	Glu	Pro	Val	Cys
	770				775					780					
Leu	Pro	Gln	Glu	Thr	Glu	Pro	Val	Val	Ser	Gly	His	Pro	Pro	Ile	Ser
785					790					795					800
Pro	His	Phe	Gly	Thr	Thr	Thr	Val	Ile	Ser	Glu	Ser	Thr	Tyr	Pro	Ser
				805					810					815	
Gly	Pro	Gly	Val	Leu	His	Pro	Lys	Pro	Ile	Leu	Asp	Pro	Leu	Gly	Tyr
				820				825					830		
Gly	Asn	Val	Thr	Val	Thr	Glu	Ser	Tyr	Thr	Thr	Ser	Asp	Thr	Leu	Lys
		835				840					845				
Pro	Ser	Val	His	Val	His	Asp	Asn	Arg	Pro	Ala	Ser	Asn	Val	Val	Val
	850														

Thr Ala Ser Ile Gly His Met Arg Ser Ser Ser Asp His His Phe Asn
 1010 1015 1020
 Gln Thr Ile Gly Ser Ala Ser Pro Ser Thr Ala Arg Ser Arg Ile Thr
 1025 1030 1035 1040
 Lys Tyr Ser Thr Val Gln Tyr Ser Lys
 1045

<210> 3
 <211> 1117
 <212> PRT
 <213> Homo Sapien

<220>
 <221> DOMAIN
 <222> (49)...(608)
 <223> Extracellular domain

<400> 3
 Met Ala Arg Thr Arg Asp Arg Val Arg Leu Leu Leu Leu Leu Ile Cys
 1 5 10 15
 Phe Asn Val Gly Ser Gly Leu His Leu Gln Val Leu Ser Thr Arg Asn
 20 25 30
 Glu Asn Lys Leu Leu Pro Lys His Pro His Leu Val Arg Gln Lys Arg
 35 40 45
 Ala Trp Ile Thr Ala Pro Val Ala Leu Arg Glu Gly Glu Asp Leu Ser
 50 55 60
 Lys Lys Asn Pro Ile Ala Lys Ile His Ser Asp Leu Ala Glu Glu Arg
 65 70 75 80
 Gly Leu Lys Ile Thr Tyr Lys Tyr Thr Gly Lys Gly Ile Thr Glu Pro
 85 90 95
 Pro Phe Gly Ile Phe Val Phe Asn Lys Asp Thr Gly Glu Leu Asn Val
 100 105 110
 Thr Ser Ile Leu Asp Arg Glu Glu Thr Pro Phe Phe Leu Leu Thr Gly
 115 120 125
 Tyr Ala Leu Asp Ala Arg Gly Asn Asn Val Glu Lys Pro Leu Glu Leu
 130 135 140
 Arg Ile Lys Val Leu Asp Ile Asn Asp Asn Glu Pro Val Phe Thr Gln
 145 150 155 160
 Asp Val Phe Val Gly Ser Val Glu Glu Leu Ser Ala Ala His Thr Leu
 165 170 175
 Val Met Lys Ile Asn Ala Thr Asp Ala Asp Glu Pro Asn Thr Leu Asn
 180 185 190
 Ser Lys Ile Ser Tyr Arg Ile Val Ser Leu Glu Pro Ala Tyr Pro Pro
 195 200 205
 Val Phe Tyr Leu Asn Lys Asp Thr Gly Glu Ile Tyr Thr Thr Ser Val
 210 215 220
 Thr Leu Asp Arg Glu Glu His Ser Ser Tyr Thr Leu Thr Val Glu Ala
 225 230 235 240
 Arg Asp Gly Asn Gly Glu Val Thr Asp Lys Pro Val Lys Gln Ala Gln
 245 250 255
 Val Gln Ile Arg Ile Leu Asp Val Asn Asp Asn Ile Pro Val Val Glu
 260 265 270
 Asn Lys Val Leu Glu Gly Met Val Glu Glu Asn Gln Val Asn Val Glu
 275 280 285
 Val Thr Arg Ile Lys Val Phe Asp Ala Asp Glu Ile Gly Ser Asp Asn
 290 295 300
 Trp Leu Ala Asn Phe Thr Phe Ala Ser Gly Asn Glu Gly Gly Tyr Phe
 305 310 315 320
 His Ile Glu Thr Asp Ala Gln Thr Asn Glu Gly Ile Val Thr Leu Ile
 325 330 335
 Lys Glu Val Asp Tyr Glu Glu Met Lys Asn Leu Asp Phe Ser Val Ile

7

Lys Thr Leu Ala Glu Val Cys Leu Gly Gln Lys Ile Asp Ile Asn Lys
 835 840 845
 Glu Ile Glu Gln Arg Gln Lys Pro Ala Thr Glu Thr Ser Met Asn Thr
 850 855 860
 Ala Ser His Ser Leu Cys Glu Gln Thr Met Val Asn Ser Glu Asn Thr
 865 870 875 880
 Tyr Ser Ser Gly Ser Ser Phe Pro Val Pro Lys Ser Leu Gln Glu Ala
 885 890 895
 Asn Ala Glu Lys Val Thr Gln Glu Ile Val Thr Glu Arg Ser Val Ser
 900 905 910
 Ser Arg Gln Ala Gln Lys Val Ala Thr Pro Leu Pro Asp Pro Met Ala
 915 920 925
 Ser Arg Asn Val Ile Ala Thr Glu Thr Ser Tyr Val Thr Gly Ser Thr
 930 935 940
 Met Pro Pro Thr Thr Val Ile Leu Gly Pro Ser Gln Pro Gln Ser Leu
 945 950 955 960
 Ile Val Thr Glu Arg Val Tyr Ala Pro Ala Ser Thr Leu Val Asp Gln
 965 970 975
 Pro Tyr Ala Asn Glu Gly Thr Val Val Val Thr Glu Arg Val Ile Gln
 980 985 990
 Pro His Gly Gly Gly Ser Asn Pro Leu Glu Gly Thr Gln His Leu Gln
 995 1000 1005
 Asp Val Pro Tyr Val Met Val Arg Glu Arg Glu Ser Phe Leu Ala Pro
 1010 1015 1020
 Ser Ser Gly Val Gln Pro Thr Leu Ala Met Pro Asn Ile Ala Val Gly
 1025 1030 1035 1040
 Gln Asn Val Thr Val Thr Glu Arg Val Leu Ala Pro Ala Ser Thr Leu
 1045 1050 1055
 Gln Ser Ser Tyr Gln Ile Pro Thr Glu Asn Ser Met Thr Ala Arg Asn
 1060 1065 1070
 Thr Thr Val Ser Gly Ala Gly Val Pro Gly Pro Leu Pro Asp Phe Gly
 1075 1080 1085
 Leu Glu Glu Ser Gly His Ser Asn Ser Thr Ile Thr Thr Ser Ser Thr
 1090 1095 1100
 Arg Val Thr Lys His Ser Thr Val Gln His Ser Tyr Ser
 1105 1110 1115

<210> 4

<211> 999

<212> PRT

<213> Homo Sapien

<220>

<221> DOMAIN

<222> (50)...(615)

<223> Extracellular domain

<400> 4

Met Met Gly Leu Phe Pro Arg Thr Thr Gly Ala Leu Ala Ile Phe Val
 1 5 10 15
 Val Val Ile Leu Val His Gly Glu Leu Arg Ile Glu Thr Lys Gly Gln
 20 25 30
 Tyr Asp Glu Glu Glu Met Thr Met Gln Gln Ala Lys Arg Arg Gln Lys
 35 40 45
 Arg Glu Trp Val Lys Phe Ala Lys Pro Cys Arg Glu Gly Glu Asp Asn
 50 55 60
 Ser Lys Arg Asn Pro Ile Ala Lys Ile Thr Ser Asp Tyr Gln Ala Thr
 65 70 75 80
 Gln Lys Ile Thr Tyr Arg Ile Ser Gly Val Gly Ile Asp Gln Pro Pro
 85 90 95
 Phe Gly Ile Phe Val Val Asp Lys Asn Thr Gly Asp Ile Asn Ile Thr

	100		105		110
Ala Ile Val Asp Arg Glu Glu Thr Pro Ser Phe Leu Ile Thr Cys Arg					
115		120		125	
Ala Leu Asn Ala Gln Gly Leu Asp Val Glu Lys Pro Leu Ile Leu Thr					
130		135		140	
Val Lys Ile Leu Asp Ile Asn Asp Asn Pro Pro Val Phe Ser Gln Gln					
145		150		155	160
Ile Phe Met Gly Glu Ile Glu Glu Asn Ser Ala Ser Asn Ser Leu Val					
165		170		175	
Met Ile Leu Asn Ala Thr Asp Ala Asp Glu Pro Asn His Leu Asn Ser					
180		185		190	
Lys Ile Ala Phe Lys Ile Val Ser Gln Glu Pro Ala Gly Thr Pro Met					
195		200		205	
Phe Leu Leu Ser Arg Asn Thr Gly Glu Val Arg Thr Leu Thr Asn Ser					
210		215		220	
Leu Asp Arg Glu Gln Ala Ser Ser Tyr Arg Leu Val Val Ser Gly Ala					
225		230		235	240
Asp Lys Asp Gly Glu Gly Leu Ser Thr Gln Cys Glu Cys Asn Ile Lys					
245		250		255	
Val Lys Asp Val Asn Asp Asn Phe Pro Met Phe Arg Asp Ser Gln Tyr					
260		265		270	
Ser Ala Arg Ile Glu Glu Asn Ile Leu Ser Ser Glu Leu Leu Arg Phe					
275		280		285	
Gln Val Thr Asp Leu Asp Glu Glu Tyr Thr Asp Asn Trp Leu Ala Val					
290		295		300	
Tyr Phe Phe Thr Ser Gly Asn Glu Gly Asn Trp Phe Glu Ile Gln Thr					
305		310		315	320
Asp Pro Arg Thr Asn Glu Gly Ile Leu Lys Val Val Lys Ala Leu Asp					
325		330		335	
Tyr Glu Gln Leu Gln Ser Val Lys Leu Ser Ile Ala Val Lys Asn Lys					
340		345		350	
Ala Glu Phe His Gln Ser Val Ile Ser Arg Tyr Arg Val Gln Ser Thr					
355		360		365	
Pro Val Thr Ile Gln Val Ile Asn Val Arg Glu Gly Ile Ala Phe Arg					
370		375		380	
Pro Ala Ser Lys Thr Phe Thr Val Gln Lys Gly Ile Ser Ser Lys Lys					
385		390		395	400
Leu Val Asp Tyr Ile Leu Gly Thr Tyr Gln Ala Ile Asp Glu Asp Thr					
405		410		415	
Asn Lys Ala Ala Ser Asn Val Lys Tyr Val Met Gly Arg Asn Asp Gly					
420		425		430	
Gly Tyr Leu Met Ile Asp Ser Lys Thr Ala Glu Ile Lys Phe Val Lys					
435		440		445	
Asn Met Asn Arg Asp Ser Thr Phe Ile Val Asn Lys Thr Ile Thr Ala					
450		455		460	
Glu Val Leu Ala Ile Asp Glu Tyr Thr Gly Lys Thr Ser Thr Gly Thr					
465		470		475	480
Val Tyr Val Arg Val Pro Asp Phe Asn Asp Asn Cys Pro Thr Ala Val					
485		490		495	
Leu Glu Lys Asp Ala Val Cys Ser Ser Ser Pro Ser Val Val Val Ser					
500		505		510	
Ala Arg Thr Leu Asn Asn Arg Tyr Thr Gly Pro Tyr Thr Phe Ala Leu					
515		520		525	
Glu Asp Gln Pro Val Lys Leu Pro Ala Val Trp Ser Ile Thr Thr Leu					
530		535		540	
Asn Ala Thr Ser Ala Leu Leu Arg Ala Gln Glu Gln Ile Pro Pro Gly					
545		550		555	560
Val Tyr His Ile Ser Leu Val Leu Thr Asp Ser Gln Asn Asn Arg Cys					
565		570		575	
Glu Met Pro Arg Ser Leu Thr Leu Glu Val Cys Gln Cys Asp Asn Arg					
580		585		590	

Gly Ile Cys Gly Thr Ser Tyr Pro Thr Thr Ser Pro Gly Thr Arg Tyr
 595 600 605
 Gly Arg Pro His Ser Gly Arg Leu Gly Pro Ala Ala Ile Gly Leu Leu
 610 615 620
 Leu Leu Gly Leu Leu Leu Leu Leu Leu Ala Pro Leu Leu Leu Thr
 625 630 635 640
 Cys Asp Cys Gly Ala Gly Ser Thr Gly Gly Val Thr Gly Gly Phe Ile
 645 650 655
 Pro Val Pro Asp Gly Ser Glu Gly Thr Ile His Gln Trp Gly Ile Glu
 660 665 670
 Gly Ala His Pro Glu Asp Lys Glu Ile Thr Asn Ile Cys Val Pro Pro
 675 680 685
 Val Thr Ala Asn Gly Ala Asp Phe Met Glu Ser Ser Glu Val Cys Thr
 690 695 700
 Asn Thr Tyr Ala Arg Gly Thr Ala Val Glu Gly Thr Ser Gly Met Glu
 705 710 715 720
 Met Thr Thr Lys Leu Gly Ala Ala Thr Glu Ser Gly Gly Ala Ala Gly
 725 730 735
 Phe Ala Thr Gly Thr Val Ser Gly Ala Ala Ser Gly Phe Gly Ala Ala
 740 745 750
 Thr Gly Val Gly Ile Cys Ser Ser Gly Gln Ser Gly Thr Met Arg Thr
 755 760 765
 Arg His Ser Thr Gly Gly Thr Asn Lys Asp Tyr Ala Asp Gly Ala Ile
 770 775 780
 Ser Met Asn Phe Leu Asp Ser Tyr Phe Ser Gln Lys Ala Phe Ala Cys
 785 790 795 800
 Ala Glu Glu Asp Asp Gly Gln Glu Ala Asn Asp Cys Leu Leu Ile Tyr
 805 810 815
 Asp Asn Glu Gly Ala Asp Ala Thr Gly Ser Pro Val Gly Ser Val Gly
 820 825 830
 Cys Cys Ser Phe Ile Ala Asp Asp Leu Asp Asp Ser Phe Leu Asp Ser
 835 840 845
 Leu Gly Pro Lys Phe Lys Lys Leu Ala Glu Ile Ser Leu Gly Val Asp
 850 855 860
 Gly Glu Gly Lys Glu Val Gln Pro Pro Ser Lys Asp Ser Gly Tyr Gly
 865 870 875 880
 Ile Glu Ser Cys Gly His Pro Ile Glu Val Gln Gln Thr Gly Phe Val
 885 890 895
 Lys Cys Gln Thr Leu Ser Gly Ser Gln Gly Ala Ser Ala Leu Ser Ala
 900 905 910
 Ser Gly Ser Val Gln Pro Ala Val Ser Ile Pro Asp Pro Leu Gln His
 915 920 925
 Gly Asn Tyr Leu Val Thr Glu Thr Tyr Ser Ala Ser Gly Ser Leu Val
 930 935 940
 Gln Pro Ser Thr Ala Gly Phe Asp Pro Leu Leu Thr Gln Asn Val Ile
 945 950 955 960
 Val Thr Glu Arg Val Ile Cys Pro Ile Ser Ser Val Pro Gly Asn Leu
 965 970 975
 Ala Gly Pro Thr Gln Leu Arg Gly Ser His Thr Met Leu Cys Thr Glu
 980 985 990
 Asp Pro Cys Ser Arg Leu Ile
 995

<210> 5

<211> 896

<212> PRT

<213> Homo Sapien

<220>

<221> DOMAIN

<222> (136)... (690)

<223> Extracellular domain

<400> 5

```

Met Ala Ala Ala Gly Pro Arg Arg Ser Val Arg Gly Ala Val Cys Leu
 1          5          10          15
His Leu Leu Leu Thr Leu Val Ile Phe Ser Arg Ala Gly Glu Ala Cys
 20          25          30
Lys Lys Val Ile Leu Asn Val Pro Ser Lys Leu Glu Ala Asp Lys Ile
 35          40          45
Ile Gly Arg Val Asn Leu Glu Glu Cys Phe Arg Ser Ala Asp Leu Ile
 50          55          60
Arg Ser Ser Asp Pro Asp Phe Arg Val Leu Asn Asp Gly Ser Val Tyr
 65          70          75          80
Thr Ala Arg Ala Val Ala Leu Ser Asp Lys Lys Arg Ser Phe Thr Ile
 85          90          95
Trp Leu Ser Asp Lys Arg Lys Gln Thr Gln Lys Glu Val Thr Val Leu
100          105          110
Leu Glu His Gln Lys Lys Val Ser Lys Thr Arg His Thr Arg Glu Thr
115          120          125
Val Leu Arg Arg Ala Lys Arg Trp Ala Pro Ile Pro Cys Ser Met
130          135          140
Gln Glu Asn Ser Leu Gly Pro Phe Pro Leu Phe Leu Gln Gln Val Glu
145          150          155          160
Ser Asp Ala Ala Gln Asn Tyr Thr Val Phe Tyr Ser Ile Ser Gly Arg
165          170          175
Gly Val Asp Lys Glu Pro Leu Asn Leu Phe Tyr Ile Glu Arg Asp Thr
180          185          190
Gly Asn Leu Phe Cys Thr Arg Pro Val Asp Arg Glu Glu Tyr Asp Val
195          200          205
Phe Asp Leu Ile Ala Tyr Ala Ser Thr Ala Asp Gly Tyr Ser Ala Asp
210          215          220
Leu Pro Leu Pro Leu Pro Ile Arg Val Glu Asp Glu Asn Asp Asn His
225          230          235          240
Pro Val Phe Thr Glu Ala Ile Tyr Asn Phe Glu Val Leu Glu Ser Ser
245          250          255
Arg Pro Gly Thr Thr Val Gly Val Val Cys Ala Thr Asp Arg Asp Glu
260          265          270
Pro Asp Thr Met His Thr Arg Leu Lys Tyr Ser Ile Leu Gln Gln Thr
275          280          285
Pro Arg Ser Pro Gly Leu Phe Ser Val His Pro Ser Thr Gly Val Ile
290          295          300
Thr Thr Val Ser His Tyr Leu Asp Arg Glu Val Val Asp Lys Tyr Ser
305          310          315          320
Leu Ile Met Lys Val Gln Asp Met Asp Gly Gln Phe Phe Gly Leu Ile
325          330          335
Gly Thr Ser Thr Cys Ile Ile Thr Val Thr Asp Ser Asn Asp Asn Ala
340          345          350
Pro Thr Phe Arg Gln Asn Ala Tyr Glu Ala Phe Val Glu Glu Asn Ala
355          360          365
Phe Asn Val Glu Ile Leu Arg Ile Pro Ile Glu Asp Lys Asp Leu Ile
370          375          380
Asn Thr Ala Asn Trp Arg Val Asn Phe Thr Ile Leu Lys Gly Asn Glu
385          390          395          400
Asn Gly His Phe Lys Ile Ser Thr Asp Lys Glu Thr Asn Glu Gly Val
405          410          415
Leu Ser Val Val Lys Pro Leu Asn Tyr Glu Glu Asn Arg Gln Val Asn
420          425          430
Leu Glu Ile Gly Val Asn Asn Glu Ala Pro Phe Ala Arg Asp Ile Pro
435          440          445
Arg Val Thr Ala Leu Asn Arg Ala Leu Val Thr Val His Val Arg Asp
450          455          460

```


Leu Asp Glu Gly Pro Glu Cys Thr Pro Ala Ala Gln Tyr Val Arg Ile
 465 470 475 480
 Lys Glu Asn Leu Ala Val Gly Ser Lys Ile Asn Gly Tyr Lys Ala Tyr
 485 490 495
 Asp Pro Glu Asn Arg Asn Gly Asn Gly Leu Arg Tyr Lys Lys Leu His
 500 505 510
 Asp Pro Lys Gly Trp Ile Thr Ile Asp Glu Ile Ser Gly Ser Ile Ile
 515 520 525
 Thr Ser Lys Ile Leu Asp Arg Glu Val Glu Thr Pro Lys Asn Glu Leu
 530 535 540
 Tyr Asn Ile Thr Val Leu Ala Ile Asp Lys Asp Asp Arg Ser Cys Thr
 545 550 555 560
 Gly Thr Leu Ala Val Asn Ile Glu Asp Val Asn Asp Asn Pro Pro Glu
 565 570 575
 Ile Leu Gln Glu Tyr Val Val Ile Cys Lys Pro Lys Met Gly Tyr Thr
 580 585 590
 Asp Ile Leu Ala Val Asp Pro Asp Glu Pro Val His Gly Ala Pro Phe
 595 600 605
 Tyr Phe Ser Leu Pro Asn Thr Ser Pro Glu Ile Ser Arg Leu Trp Ser
 610 615 620
 Leu Thr Lys Val Asn Asp Thr Ala Ala Arg Leu Ser Tyr Gln Lys Asn
 625 630 635 640
 Ala Gly Phe Gln Glu Tyr Thr Ile Pro Ile Thr Val Lys Asp Arg Ala
 645 650 655
 Gly Gln Ala Ala Thr Lys Leu Leu Arg Val Asn Leu Cys Glu Cys Thr
 660 665 670
 His Pro Thr Gln Cys Arg Ala Thr Ser Arg Ser Thr Gly Val Ile Leu
 675 680 685
 Gly Lys Trp Ala Ile Leu Ala Ile Leu Leu Gly Ile Ala Leu Leu Phe
 690 695 700
 Ser Val Leu Leu Thr Leu Val Cys Gly Val Phe Gly Ala Thr Lys Gly
 705 710 715 720
 Lys Arg Phe Pro Glu Asp Leu Ala Gln Gln Asn Leu Ile Ile Ser Asn
 725 730 735
 Thr Glu Ala Pro Gly Asp Asp Arg Val Cys Ser Ala Asn Gly Phe Met
 740 745 750
 Thr Gln Thr Thr Asn Asn Ser Ser Gln Gly Phe Cys Gly Thr Met Gly
 755 760 765
 Ser Gly Met Lys Asn Gly Gly Gln Glu Thr Ile Glu Met Met Lys Gly
 770 775 780
 Gly Asn Gln Thr Leu Glu Ser Cys Arg Gly Ala Gly His His His Thr
 785 790 795 800
 Leu Asp Ser Cys Arg Gly Gly His Thr Glu Val Asp Asn Cys Arg Tyr
 805 810 815
 Thr Tyr Ser Glu Trp His Ser Phe Thr Gln Pro Arg Leu Gly Glu Lys
 820 825 830
 Leu His Arg Cys Asn Gln Asn Glu Asp Arg Met Pro Ser Gln Asp Tyr
 835 840 845
 Val Leu Thr Tyr Asn Tyr Glu Gly Arg Gly Ser Pro Ala Gly Ser Val
 850 855 860
 Gly Cys Cys Ser Glu Lys Gln Glu Glu Asp Gly Leu Asp Phe Leu Asn
 865 870 875 880
 Asn Leu Glu Pro Lys Phe Ile Thr Leu Ala Glu Ala Cys Thr Lys Arg
 885 890 895

<210> 6

<211> 31

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIVF

<400> 6

Asp	Lys	Ile	Cys	Leu	Gly	His	His	Ala	Val	Ser	Asn	Gly	Thr	Lys	Val
1			5						10					15	
Asn	Thr	Leu	Thr	Glu	Arg	Gly	Val	Glu	Val	Val	Asn	Ala	Thr	Glu	
		20						25					30		

<210> 7

<211> 41

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIVH

<400> 7

Gln	Asp	Leu	Pro	Gly	Asn	Asp	Asn	Asn	Thr	Ala	Thr	Leu	Cys	Leu	Gly
1				5					10					15	
His	His	Ala	Val	Pro	Asn	Gly	Thr	Leu	Val	Lys	Thr	Ile	Thr	Asp	Asp
			20					25					30		
Gln	Ile	Glu	Val	Thr	Asn	Ala	Thr	Glu							
		35					40								

<210> 8

<211> 41

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIV6

<400> 8

Gln	Asn	Leu	Pro	Gly	Asn	Asp	Asn	Ser	Thr	Ala	Thr	Leu	Cys	Leu	Ala
1				5					10					15	
His	His	Ala	Val	Pro	Asn	Gly	Thr	Leu	Val	Lys	Thr	Ile	Thr	Asn	Asp
			20					25					30		
Gln	Ile	Glu	Val	Thr	Asn	Ala	Thr	Glu							
		35					40								

<210> 9

<211> 42

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIVV

<400> 9

Gln	Asp	Leu	Pro	Gly	Asn	Asp	Asn	Asn	Ser	Thr	Ala	Thr	Leu	Cys	Leu
1				5					10					15	
Gly	His	His	Ala	Val	Pro	Asn	Gly	Thr	Leu	Val	Lys	Thr	Ile	Thr	Asn
			20					25					30		
Asp	Gln	Ile	Glu	Val	Thr	Asn	Ala	Thr	Glu						
		35					40								

<210> 10

<211> 41

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIVDU

<400> 10

Gln	Asp	Leu	Pro	Gly	Asn	Asp	Asn	Ser	Thr	Ala	Thr	Leu	Cys	Leu	Gly
1				5				10					15		
His	His	Ala	Val	Pro	Asn	Gly	Thr	Ile	Val	Lys	Thr	Ile	Thr	Asp	Asp
		20					25					30			
Gln	Ile	Glu	Val	Thr	Asn	Ala	Thr	Glu							
	35						40								

<210> 11

<211> 41

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIVH3

<400> 11

Gln	Tyr	Leu	Pro	Gly	Asn	Asp	Asn	Ser	Thr	Ala	Thr	Leu	Cys	Leu	Gly
1				5				10					15		
His	His	Ala	Val	Pro	Asn	Gly	Thr	Leu	Val	Lys	Thr	Ile	Thr	Asn	Asp
		20					25					30			
Gln	Ile	Glu	Val	Thr	Asn	Ala	Thr	Glu							
	35						40								

<210> 12

<211> 31

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIVH7

<400> 12

Asp	Lys	Ile	Cys	Leu	Gly	His	His	Ala	Val	Ala	Asn	Gly	Thr	Lys	Val
1			5					10					15		
Asn	Thr	Leu	Thr	Glu	Arg	Gly	Ile	Glu	Val	Val	Asn	Ala	Thr	Glu	
		20					25					30			

<210> 13

<211> 31

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIV10

<400> 13

Asp	Lys	Ile	Cys	Leu	Gly	His	His	Ala	Val	Pro	Asn	Gly	Ile	Ile	Val
1			5					10					15		
Lys	Thr	Leu	Thr	Asn	Glu	Lys	Glu	Glu	Val	Thr	Asn	Ala	Thr	Glu	
		20					25					30			

<210> 14

<211> 35

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIVH6

<400> 14

Asp	Lys	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Thr	Gln	Ile
1				5					10					15	
Asp	Thr	Ile	Leu	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ser	Val	Glu	Leu
			20					25					30		
Leu	Glu	Asn													
		35													

<210> 15

<211> 35

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIV5

<400> 15

Asp	Thr	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Asp	Thr	Val
1				5					10					15	
Asp	Thr	Ile	Phe	Glu	Lys	Asn	Val	Ala	Val	Thr	His	Ser	Val	Asn	Leu
			20					25					30		
Leu	Glu	Asp													
		35													

<210> 16

<211> 35

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIV

<400> 16

Asp	Thr	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Asp	Thr	Val
1				5					10					15	
Asp	Thr	Val	Leu	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ser	Val	Asn	Leu
			20					25					30		
Leu	Glu	Asp													
		35													

<210> 17

<211> 35

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIVUR

<400> 17

Asp	Thr	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Asp	Thr	Val
1				5					10					15	
Asp	Thr	Val	Leu	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ser	Val	Asn	Leu
			20					25					30		
Leu	Glu	Asp													
		35													

<210> 18

<211> 35

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIV2

<400> 18

Asp	Gln	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Glu	Lys	Val
1				5					10					15	
Asp	Thr	Asn	Leu	Glu	Arg	Asn	Val	Thr	Val	Thr	His	Ala	Lys	Asp	Ile
			20					25					30		
Leu	Glu	Lys													
		35													

<210> 19

<211> 35

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIVH2

<400> 19

Asp	Gln	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Glu	Lys	Val
1				5					10					15	
Asp	Thr	Ile	Leu	Glu	Arg	Asn	Val	Thr	Val	Thr	His	Ala	Lys	Asp	Ile
			20					25					30		
Leu	Glu	Lys													
		35													

<210> 20

<211> 35

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIVH5

<400> 20

Asp	Gln	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Glu	Gln	Val
1				5					10					15	
Asp	Thr	Ile	Met	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ala	Gln	Asp	Ile
			20					25					30		
Leu	Glu	Lys													
		35													

<210> 21

<211> 35

<212> PRT

<213> Influenza Strain A

<220>

<223> HMIVN1

<400> 21

Asp	Thr	Leu	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Asp	Thr	Val
1				5					10					15	
Asp	Thr	Val	Leu	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ser	Val	Asn	Leu
			20					25					30		
Leu	Glu	Asp													
		35													

<210> 22
<211> 12
<212> PRT
<213> Chicken bFGF-R

<400> 22
Glu Lys Met Glu Lys Lys Leu His Ala Val Pro Ala
1 5 10

<210> 23
<211> 12
<212> PRT
<213> Mouse FGF-R

<220>
<223> i

<400> 23
Glu Lys Met Glu Lys Lys Leu His Ala Val Pro Ala
1 5 10

<210> 24
<211> 12
<212> PRT
<213> Frog FGF-R

<220>
<223> i

<400> 24
Glu Lys Met Glu Lys Lys Leu His Ala Val Pro Ala
1 5 10

<210> 25
<211> 12
<212> PRT
<213> Mouse KGF-R

<220>
<223> i

<400> 25
Glu Lys Met Glu Lys Arg Leu His Ala Val Pro Ala
1 5 10